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Fish community responses to metal pollution

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The IBI can be used to assess the effect of metals on fish community structure.

Abstract

The effect of metal pollution on fish communities was assessed at 47 sites, including a Cd and Zn gradient. Fish community structure was assessed by applying diversity indices, index of biotic integrity and the abundance/biomass comparison method (ABC-index). To relate the community responses to metal pollution, toxic unit values for dissolved metals (TU_w) and for metals in fish liver (TU_t) were calculated. If all sites were considered only a good relationship was found between the IBI and the TU_w, with 56% of the variation in IBI being described. Within the pollution gradient (sites 1–12) the described variation increased up to 85%. Relating the community structure to TU_t, the described variation in IBI further increased to 87%. Our results indicate that besides water quality other factors influenced diversity and ABC-index. The IBI proved a suitable index for the assessment of metal pollution, provided that other water quality characteristics meet the water quality criteria.
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Keywords: Diversity indices; Trace metals; Fish community; Toxic units; IBI; ABC-index

1. Introduction

Fish community structure has been widely used to assess the effect of human impacts on aquatic ecosystems including water quality deterioration and habitat changes (Karr, 1981; Maret et al., 1997; Wolter et al., 2000; Angermeier and Davideanu, 2004; Pirhalla, 2004). Few studies, however, investigated the effect of a single specific stressor such as metal pollution on the structure of fish communities (Dyer et al., 2000a). Yet trace metals pose a serious threat to the survival of freshwater fish. High levels of metals discharged in aquatic ecosystems might result in selective elimination of the most sensitive life stages of vulnerable fish species. Chronic exposure of fish to sublethal trace metal levels causes, among others, disturbed ion regulation, reduced swimming speed and reduced growth and condition (Sörensen, 1991; Hollis et al., 1999; Alsop et al., 1999; Bervoets and Blust, 2003). As a consequence it can be expected that metal pollution will also result in alterations of the fish community, both in species composition and richness and trophic composition. However, it is very difficult to investigate fish community responses to metal pollution since only a few aquatic systems can be found where heavy metal pollution is the only major anthropogenic impact.

The characteristics of fish communities including species diversity, total biomass and length frequency distribution can be considered as highly relevant endpoints since they reflect the health of the whole aquatic habitat, including habitat quality, food availability and water quality (Smith et al., 1999; Kovacs et al., 2002; MacDonald et al., 2002). A method frequently used for the assessment of fish community

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structure is the multi-metric index of biotic integrity (IBI). The IBI was first introduced by Karr (1981) and is based on fish assemblage characteristics, such as species diversity, trophic composition and fish biomass. Since 1981 the IBI has been applied and adapted for different river systems in different countries (Kamdem-Toham and Teugels, 1999; Belpaire et al., 2000; Angermeier and Davideanu, 2004; Pirhalla, 2004). Belpaire et al. (2000) illustrated the applicability of the IBI for the assessment of ecological quality of Flemish water bodies. In several studies the IBI has been used to assess the impact of water pollution (Hartwell, 1997; Lydy et al., 2000; Dyer et al., 2000b; Kovacs et al., 2002; Porter and Janz, 2003). Another promising method to assess the quality of fish communities is the abundance/biomass comparison method (ABC-method). The ABC-method has been developed by Warwick (1986) as a method to assess the effect of water pollution on communities of marine macro-invertebrates. This method is based on the theoretical assumption that under 'stable' conditions interspecific competition will result in a relatively low diversity equilibrium. Under these conditions the competitive species are *k*-selected with the attributes of a large body size, long lifespan and a fairly constant population size. Under 'disturbed' conditions, no competitive equilibrium is reached, diversity increases and the dominant species are *r*-selected species with a small body size and short lifespans. Although the ABC-method has been primarily applied to macro benthos communities (Warwick et al., 1987; Beukema, 1988; Warwick and Clarke, 1994; Simboura et al., 1995; Harkantra and Rodrigues, 2004) it also has been used on birds (Meire and Dereu, 1990) and marine and freshwater fish communities (Coeck et al., 1993; Penczak and Kruk, 1999; Barletta et al., 2003).

In Flanders (northern part of Belgium), water quality has improved significantly over the last 20 years as a result of increased purification of municipal wastewater. This has resulted in increased oxygen levels, decreased organic loads and (partially) recovered fish communities in several river systems (Vandamme et al., 1994; Vandelannoote et al., 1998; De Cooman et al., 2003). On the other hand, heavy metal pollution substantially increased in the last decades in many watercourses, representing a significant risk to the aquatic biota. For example, in the Molse Nete–Grote Nete river system, which belongs to the river Scheldt basin, a pronounced downstream concentration gradient of Cd and Zn can be observed in both water and fish tissue (gudgeon; *Gobio gobio*) samples (Bervoets and Blust, 2003). Since river characteristics (i.e. water hardness, depth, width, current speed) remain relatively constant along this gradient, this river system represents an ideal study area to investigate the impact of metal pollution on fish communities.

The aim of this study was to investigate the fish community structure at several watercourses in Flanders with different levels of metal pollution, including a metal pollution gradient (the Molse Nete–Grote Nete River system). Applying different approaches, we examined if changes in the fish community structure in these river systems could be related to changes in environmental and/or accumulated metal levels. Accumulated levels were obtained from metal analysis in the liver of the gudgeon, captured at the different sites.

In a first approach fish diversity was assessed. The second approach involved the characterisation of the community structure with an index of biotic integrity. In the third approach the abundance/biomass method was used for the characterisation of the fish fauna.

2. Materials and methods

2.1. Study area and sampling

Forty-seven sampling sites, characterised by different levels of metal contamination, were selected in the Scheldt and Meuse river basin in Flanders, Belgium (Table 1, Fig. 1). These locations included 12 sites along a pronounced metal pollution gradient in the Molse Nete–Grote Nete river system (sites 1–12, Fig. 1). Cd and Zn concentrations in the water at the upstream part of this river system are currently in excess of Belgian and international water quality objectives (1 μg/l Cd; 200 μg/l Zn; Bervoets and Blust, 2003). All sites under investigation were characterised by high levels of oxygen and low levels of organic pollution (Flemish Environmental Agency, http://www.vmm.be/geoview/geoinfo/fysico-chemie).

Water samples were collected in duplicate each month between January and July 2001 at all sampling locations. One sample was filtered through a 0.45 μm Millipore membrane filter and acidified with 1 M ultrapure nitric acid (to a pH of 2.0) and stored in 20 ml acid washed polypropylene vials at 4 °C until metal analysis. The other water sample was analysed within 24 h on a set of water characteristics (see further).

At all sampling sites fish were caught between December 2000 and January 2001 by electrofishing, using an Electra catch WFC7 generator producing 150 V. Three successive samplings were conducted at each site (length: ±100 m). All fish were identified to the species level and counted. For all individuals, fork length was measured (±1 mm) and weight determined using a Kern 442.43 balance (±0.1 g). Population densities (individuals/ha) and biomass (kg/ha) of each species at each location were estimated by the depletion method (Laurent and Lamarque, 1974; Penczak et al., 2003).

At sites 1–23, 8–10 individuals of gudgeon (*G. gobio*) were sacrificed using the anaesthetic ethyl *meta*-aminobenzoate methanesulphonic acid (MS222) and tissues

Table 1
Location of the different sampling sites

Site	Name and place	River basin	Location
1	ScheppelijkeNete	Nete/Scheldt	Balen
2	Scheppelijke Nete	Nete/Scheldt	Balen
3	Scheppelijke Nete	Nete/Scheldt	Mol
4	Scheppelijke Nete	Nete/Scheldt	Mol
5	Molse Nete	Nete/Scheldt	Mol
6	Molse Nete	Nete/Scheldt	Mol
7	Grote Nete	Nete/Scheldt	Geel
8	Grote Nete	Nete/Scheldt	Geel
9	Grote Nete	Nete/Scheldt	Westerlo
10	Grote Nete	Nete/Scheldt	Westmeerbeek
11	Grote Nete	Nete/Scheldt	Kessel
12	Grote Nete	Nete/Scheldt	Lier
13	Molse Nete	Nete/Scheldt	Balen
14	Brisdiloop	Nete/Scheldt	Leopoldsburg
15	Asbeek	Nete/Scheldt	Balen
16	Kneutersloop	Nete/Scheldt	Olen
17	Diepteloop	Nete/Scheldt	Beerse
18	Wimp	Nete/Scheldt	Herenthout
19	Desselse Nete 1	Nete/Scheldt	Dessel
20	Witte Nete	Nete/Scheldt	Dessel
21	Voorste Nete	Nete/Scheldt	Dessel
22	Oude Nete	Nete/Scheldt	Mol
23	Grote Nete	Nete/Scheldt	Meerhout
24	Breiloop 1	Nete/Scheldt	Kasterlee
25	Voer	Meuse	Voeren
26	Voer	Meuse	Voeren
27	Veurs	Meuse	Voeren
28	Groot Schijn	Schijn/Scheldt	Oelegem
29	Zevenborrebeek	Zenne/Scheldt	Dworp
30	Berwijn	Meuse	Moelingen
31	Laarse Beek	Scheldt	Brasschaat
32	Steenputbeek	Zenne/Scheldt	Dworp
33	Merkske	Mark/Meuse	Hoogstraten
34	Desselse Nete 2	Nete/Scheldt	Dessel
35	Ruwaal	Dijle/Scheldt	Korbeek-Dijle
36	Verrebeek	Zwalm/Scheldt	Brakel
37	Breiloop 2	Nete/Scheldt	Kasterlee
38	Molenbeek	Zwalm/Scheldt	Maarkedal
39	Nethen 1	Dijle/Scheldt	Sint-Joris-Weert
40	Nethen 2	Dijle/Scheldt	Sint-Joris-Weert
41	Paddepoel	Dijle/Scheldt	Sint-Joris-Weert
42	Tappelbeek	Nete/Scheldt	Zandhoven
43	Zwanebeek	Schijn/Scheldt	Schilde
44	Zwarte beek	Demer/Scheldt	Beringen
45	Vleterbeek	Ijzer	Poperinge
46	Aabeek	Meuse	Kinrooi
47	Bosbeek	Nete/Scheldt	Vorselaar

(kidney, gill, liver and muscle) were dissected in the field. The organs were stored in liquid nitrogen and transported to the laboratory. Samples for metal analysis were put in acid washed polypropylene pre-weighted vials and dried for 24 h at 60 °C. Subsequently, the biological material was digested with concentrated ultrapure nitric acid in a microwave oven and stored until analysis (Bervoets and Blust, 2003).

2.2. Chemical measurements

Water quality characteristics (pH; temperature (°C); conductivity (μS/cm); oxygen (mg/l)) were measured on-site and at each sampling moment with a WTW multiline F/SET-3 field kit. Chloride, sulphate, phosphate, ammonium, nitrate and nitrite were determined using an automated analyser (Skalar: FAS, SA 20/40). Biological oxygen demand (BOD_5^{20}) was determined by incubating the samples at 20 °C during 5 days and remeasuring the oxygen content.

Cadmium, cobalt, chromium, copper, nickel, lead and zinc were measured in the water and fish tissue samples with either an inductively coupled plasma-atomic emission spectrometer (ICP-AES, Varian Liberty Series II, Victoria, Australia) equipped with a micro-concentric groove nebulizer (De Wit and Blust, 1998) or an inductively coupled plasma-mass spectrophotometer (ICP-MS, Varian UltraMass 700, Victoria, Australia).

2.3. Fish community assessment

Two indices of species diversity were calculated: Menhinick (I) and Shannon (H):

$$I = S/\sqrt{N}$$

$$H = -\sum p_i \ln p_i$$

where S is the number of species, N the number of individuals and p_i is the percentage of individuals of a given species in the total number of individuals.

The index of biotic integrity (IBI) was calculated according to Belpaire et al. (2000). For the type of watercourses under investigation, the IBI is comprised of eight metrics each scored from 1 to 5. Fish metrics that were determined are total number of species, mean tolerance, mean typical species value, relative presence of type species, total biomass (kg/ha), weight % of non-native species, trophic composition and relative natural recruitment (Belpaire et al., 2000; Breine et al., 2004). The overall IBI score for a given sampling site was calculated as the mean of scores for all metrics and ranging from 0 to 5.

Since the abundance/biomass method (ABC) is based on the theoretical considerations that the distribution of numbers of individuals among species differs from the distribution of their biomass when influenced by pollution (Warwick, 1986), these differences can be shown by combined k-dominance plots (Lambshead et al., 1983). In order to make comparison among sampling sites and with environmental metal pollution possible, an ABC-index was calculated according to Meire and Dereu (1990):

$$\mathrm{ABC} = \sum_{i=1}^{N} (B_i - A_i)/N$$

where B_i is the percentage dominance of species i (ranked from the highest to the lowest biomass) and A_i

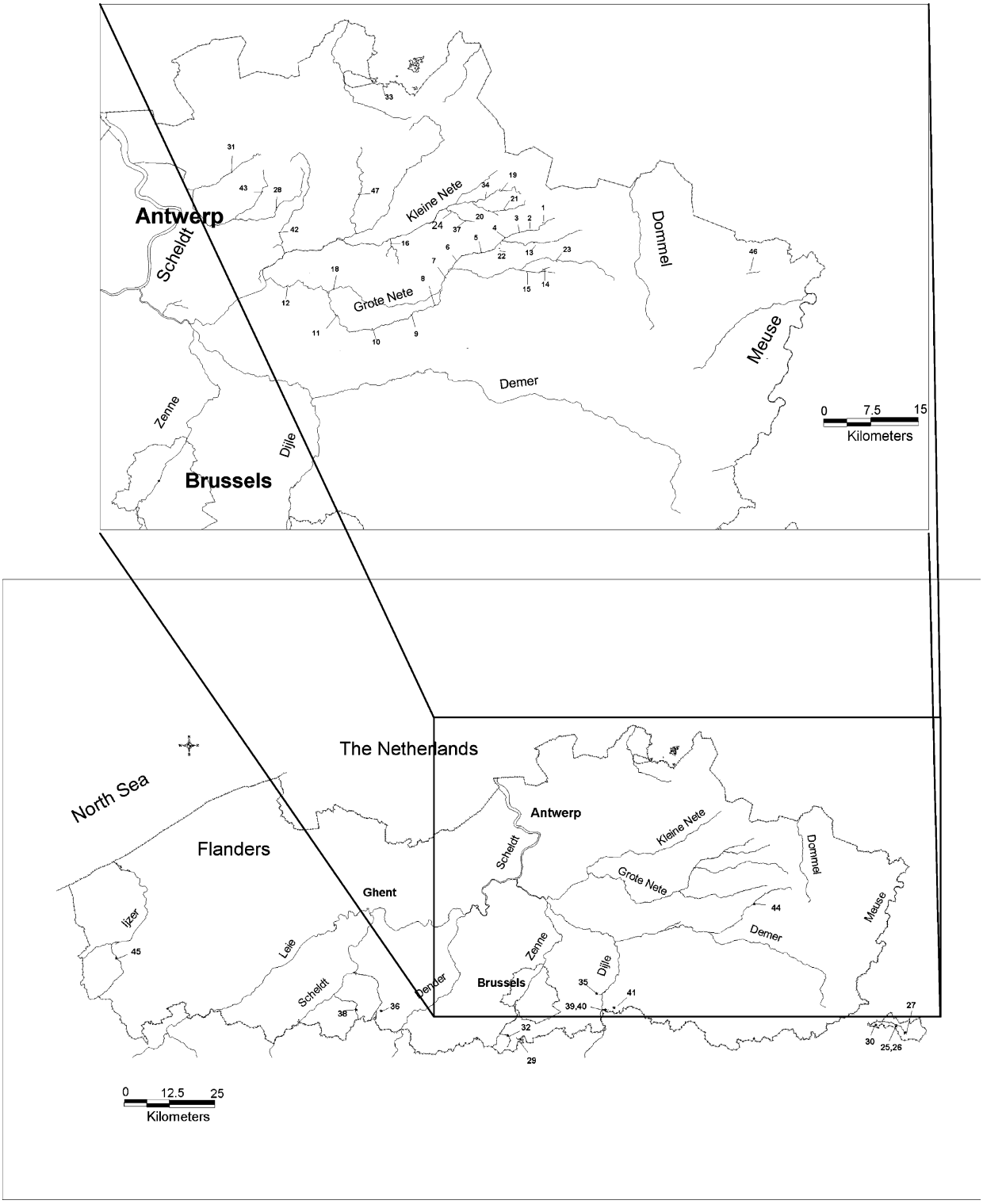

Fig. 1. Map of the sampling sites. Samples were collected from 47 sites in winter 2000–2001.

is the percentage dominance of species i (ranked from the most to the least abundant species).

2.4. Relationship between fish community structure and pollution load

To relate fish community responses to metal contamination, data on dissolved metal levels at each study site were converted to toxic units (TU_w). The TU_w of a compound is defined as the concentration of the compound divided by the water quality criteria of that compound. The TU_w of the different compounds were summed for each sampling location (Doi, 1994). Water quality standards were derived from the US Environmental Protection Agency (1999). At site 1–23, metal levels were also measured in tissues of the gudgeon.

Table 2
General water quality characteristics at the different sampling sites

Site	O_2 (mg/l)	pH	BOD (mg/l)	SO_4^{2-} (mg/l)	Cl^- (mg/l)	$NO_3^- + NO_2^-$-N	NH_4^+-N (mg/l)	PO_4^{3-}-P (mg/l)
1	8.2	7.2	1.8	82	51	2.2	0.26	0.05
2	9.3	7.2	0.50	85	42	2.0	0.23	0.05
3	8.5	7.1	1.2	77	39	1.7	0.41	0.08
4	8.6	7.2	2.6	95	38	1.6	0.35	0.09
5	9.3	7.3	4.9	61	37	1.5	0.68	0.11
6	8.7	7.3	8.8	57	36	2.6	0.88	0.16
7	10.1	7.3	4.9	49	30	2.1	0.52	0.14
8	10.9	7.6	6.9	50	40	2.2	1.2	0.18
9	10.6	7.4	5.1	55	365	1.8	1.2	0.13
10	10.4	7.4	4.1	55	313	1.9	1.1	0.08
11	9.8	7.5	5.1	57	201	1.7	1.1	0.12
12	9.8	7.4	4.0	58	156	1.7	0.92	0.12
13	9.2	7.3	5.7	66	34	2.3	0.68	0.13
14	8.5	6.4	ND	34	19	1.3	0.26	0.07
15	9.9	6.7	3.0	32	25	1.1	0.26	0.10
16	6.5	7.0	3.4	823	534	2.2	4.0	0.17
17	9.8	6.9	2.8	93	39	1.9	1.0	0.20
18	7.6	6.8	7.0	60	53	1.6	2.2	0.13
19	9.7	7.0	2.6	54	36	1.5	0.09	0.03
20	9.8	7.5	2.3	54	34	1.6	0.17	0.04
21	8.9	6.9	2.2	57	35	2.5	0.29	0.02
22	8.7	7.0	ND	ND	ND	ND	ND	ND
23	8.3	6.8	2.0	33	21	1.3	0.33	0.16
24	10.6	6.9	3.2	82	48	1.7	0.34	0.07
25	12.0	8.0	2.9	31	16	8.1	0.16	0.07
26	11.1	8.4	2.4	33	17	9.0	0.10	0.07
27	10.7	8.0	2.1	44	15	7.4	0.16	0.06
28	7.3	7.4	7.0	67	29	0.65	1.1	0.05
29	8.5	8.0	3.3	59	36	3.6	0.15	0.03
30	11	8.1	4.3	59	22	5.8	0.18	0.19
31	6.6	7.3	3.6	76	32	0.45	0.37	0.03
32	10.4	8.1	3.0	67	36	4.5	0.14	0.04
33	9.6	7.2	2.9	69	29	5.3	0.15	0.06
34	10.1	7.0	2.3	55	34	1.6	0.16	0.04
35	9.7	8.0	4.0	62	36	6.6	0.23	0.09
36	10.3	7.9	3.1	62	25	2.5	0.08	0.06
37	8.6	7.0	2.4	76	50	1.9	0.30	0.06
38	10.6	7.9	5.6	82	43	5.9	0.54	0.17
39	9.2	8.0	6.9	68	42	5.0	0.89	0.25
40	7.0	7.8	7.2	69	43	5.2	1.0	0.25
41	9.2	8.1	3.2	66	19	1.4	0.22	0.11
42	6.9	7.3	4.9	68	28	0.57	0.61	0.07
43	8.1	7.6	4.2	62	18	0.22	0.44	0.05
44	9.9	7.2	2.5	33	14	3.6	0.14	0.07
45	9.1	7.9	7.6	136	60	12	0.76	0.21
46	8.8	7.5	4.0	49	31	0.83	0.49	0.10
47	7.5	7.0	3.0	60	29	0.49	0.32	0.06
CQC USEPA	5.5–9.5	6.5–9.0	NA	500	250	NA	[a]	NA
FQC	≥5.0	6.5–8.0	≤6.0	250	200	10.0	1.0	0.30

CQC, Canadian quality criteria for aquatic freshwater life; USEPA, US Environmental Protection Agency; FQC, Flemish quality criteria; NA, not available; ND, not determined.
[a] pH dependent.

Tissue toxic units (TU_t) were calculated using the formula

$$TU_t = \frac{[\sum_i (C_{ij}/CR_i)]}{N}$$

where TU_t is the metal load in the liver of the gudgeon at a site, C_{ij} is the concentration of metal i at site j, CR_i is the concentration of metal i at the reference site and N is the number of metals measured (Dyer et al., 2000b; Meregalli et al., 2000; Bervoets and Blust, 2003). When $C_{ij} < CR_i$, C_{ij}/CR_i was considered 1. Thus the TU_t is a measure of the enrichment of the liver with the measured metals, compared to those in fish from unpolluted sites. As a consequence, when no enrichment occurred, $TU_t = 1$. Metal levels in gudgeon liver from unpolluted sites were obtained from Bervoets and Blust (2003).

2.5. Statistical analysis

Linear and non-linear regressions were used to analyse the data. Significance levels of tests are indicated by asterisks according to the following probability ranges: $^{*}p \leq 0.05$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. Statistical methods used are outlined in Sokal and Rohlf (1998). All statistical analyses were performed using the software package STATISTICA (StatSoft Inc., Tulsa).

3. Results

3.1. Water quality characteristics

General water quality characteristics of the different sampling sites are summarised in Table 2. These results clearly show that all sites had good water quality characteristics, with the exception of chloride at sites 9–12 and site 16, and ammonium at sites 8–11, if compared to the national and international water quality standards (Canadian Council of Ministers of the Environment, 1999; Flemish Government, 2000; US Environmental Protection Agency, 1999).

Levels of measured dissolved metals are shown in Table 3. At sites 1–16, all belonging to the Nete river system, dissolved levels of one or more metals exceeded the water quality criteria. High to very high levels of Cd and Zn were measured in the metal pollution gradient running from site 2 to site 12. At sites 1 and 13, Cd and Zn levels were also high. Furthermore, high levels of Cd were measured at sites 14, 15 and 16 and very high levels of Ni at site 16. Water quality criteria were not exceeded at any of the other sites.

Table 3
Dissolved metal levels at the sampling sites

Site	Cd (μg/l)	Co (μg/l)	Cr (μg/l)	Cu (μg/l)	Ni (μg/l)	Pb (μg/l)	Zn (μg/l)
1	3.9	9.3	1.1	4.1	20	5.1	712
2	62	13	1.7	8.3	14	17	5539
3	38	11	1.8	6.1	17	11	3853
4	58	13	2.6	8.1	17	18	4864
5	17	7.1	2.0	5.7	12	11	1762
6	8.4	4.5	1.5	5.4	15	7.5	922
7	4.3	3.8	1.2	5.9	12	5.3	506
8	1.5	14	< 0.50	1.3	4.1	6.7	185
9	1.0	11	< 0.50	0.25	4.3	5.0	147
10	1.0	10	< 0.50	0.50	4.0	5.0	163
11	1.0	8.0	0.93	0.25	7.3	12	128
12	1.1	6.2	0.86	0.22	18	5.0	176
13	5.7	0.69	1.40	2.5	9.4	0.2	675
14	1.1	1.3	< 0.50	28	20	1.2	193
15	2.5	6.5	< 0.50	18	12	0.21	192
16	1.7	42	4.7	23	490	< 0.10	7.2
17	2.7	1.3	< 0.50	46	15	0.20	312
18	< 0.10	0.71	1.7	3.5	7.3	2.0	62
19	< 0.10	0.52	< 0.50	1.9	8.5	0.23	11
20	< 0.10	0.50	< 0.50	2.4	6.1	0.27	24
21	1.2	0.49	6.0	3.2	5.7	6.0	88
22	< 0.10	1.1	< 0.50	2.7	2.7	0.26	86
23	< 0.10	0.89	< 0.50	4.4	4.9	3.1	148
24	< 0.10	0.56	< 0.50	67	5.9	1.1	55
25	< 0.10	1.6	3.8	11	21	0.20	33
26	< 0.10	0.29	< 0.50	2.2	4.5	< 0.10	19
27	< 0.10	0.22	< 0.50	1.6	4.5	< 0.10	7.4
28	< 0.10	0.52	< 0.50	1.9	8.1	< 0.10	10
29	< 0.10	0.23	< 0.50	1.3	4.5	< 0.10	5.1
30	< 0.10	0.19	< 0.50	3.0	3.6	< 0.10	10
31	< 0.10	0.22	< 0.50	1.6	3.0	< 0.10	3.4
32	< 0.10	0.25	< 0.50	1.0	3.8	0.27	3.9
33	< 0.10	0.87	< 0.50	2.7	24	0.21	13
34	< 0.10	0.51	< 0.50	12	6.7	< 0.10	10
35	< 0.10	0.65	< 0.50	11	3.1	< 0.10	16
36	< 0.10	0.33	< 0.50	4.0	2.4	< 0.10	28
37	< 0.10	0.38	< 0.50	1.4	5.9	< 0.10	5.8
38	< 0.10	0.56	< 0.50	2.6	6.3	< 0.10	3.7
39	< 0.10	0.43	< 0.50	1.4	6.2	< 0.10	6.2
40	< 0.10	0.30	< 0.50	1.3	4.0	< 0.10	4.6
41	< 0.10	0.29	< 0.50	0.4	4.2	< 0.10	5.9
42	< 0.10	0.42	< 0.50	4.3	6.8	< 0.10	6.4
43	< 0.10	0.30	< 0.50	2.9	9.0	0.60	7.9
44	< 0.10	0.39	6.0	2.0	8.6	< 0.10	46
45	< 0.10	0.32	< 0.50	2.7	5.7	< 0.10	4.3
46	< 0.10	0.43	< 0.50	1.6	6.5	0.24	20
47	< 0.10	0.26	< 0.50	2.7	4.0	< 0.10	6.1
CQC	0.02	50	50	24	25	17	30
USEPA	2.2	NA	74	9.0	52	2.5	120
FQC	1.0	NA	50	50	30	50	200

CQC, Canadian quality criteria for aquatic freshwater life; USEPA, US Environmental Protection Agency; FQC, Flemish quality criteria; NA, not available.

3.2. Fish community structure

In total 29 species were captured, most of them belonging to the Cyprinidae (Table 4). The results of the different community structure indices at each study site are summarised in Table 5. The number of captured fish

Table 4
Fish species collected at the different sampling sites (see also Fig. 1)

Family – species	Sites of occurrence
Petromyzonidae	
Lampetra planeri (Bloch) – brook lamprey	7, 19, 25, 32, 36, 44
Aguillidae	
Anguilla anguilla (L.) – European eel	1–13, 19, 20, 24, 28–29, 34, 42, 43, 45–47
Esocidae	
Esox lucius L. – pike	5–8, 31
Umbridae	
Umbra pygmea (De Kay) – eastern mud-minnow	1, 2, 5, 22, 23, 44, 46
Salmonidae	
Salmo trutta L. – brown trout	25, 26, 30, 32
Cyprinidae	
Cyprinus carpio L. – common carp	6, 11, 12, 18, 23, 46
Abramis brama L. – bream	2, 5–7, 11, 12, 22, 31, 45
Alburnus alburnus (L.) – bleak	20
Alburnoides bibunctatus (Bloch) – spirlin	30
Barbus barbus (L.) – barbel	30
Blicca bjoerkna (L.) – silver bream	6, 10–12
Carassius auratus gibelio (Bloch) – Prussian carp	4, 6, 8–12, 18, 24, 37, 45
Gobio gobio (L.) – gudgeon	1, 4–11, 13–23, 24, 26–30, 33, 34, 37, 39–42, 44–47
Leuciscus cephalus (L.) – chub	6, 9, 18, 23, 30
Leuciscus idus (L.) – ide	5, 10–12, 28
Phoxinus phoxinus (L.) – minnow	26, 30
Pseudorasbora parva – stone moroko	1, 3, 5–7, 9, 18, 22, 23, 35, 44
Rhodeus sericeus (Bloch) – bitterling	35
Rutilus rutilus (L.) – roach	1, 3–12, 15,16, 18, 20–24, 26, 28–31, 34, 44–47
Scardinius erythrophtalmus (L.) – rudd	1, 4–6, 8, 10–12, 15, 18, 22, 33, 46
Tinca tinca (L.) – tench	24,46
Cobitidae	
Cobitis taenia L. – spined loach	19–21, 24, 28, 34
Barbatula barbatula (L.) – stone loach	1, 5–8, 14, 18–24, 30, 33–35, 37–39, 41–44, 46, 47
Ictaluridae	
Ictalurus nebulosus (Le Sueur) – brown bullhead	2, 3, 6–8, 10, 12, 46
Gasterosteidae	
Gasterosteus aculeatus L. – 3-spined stickleback	1, 5–13, 15, 17–21, 23, 24, 28–30, 33, 35, 37–42
Pungitius pungitius (L.) – 10-spined stickleback	1, 7–9, 19–22, 23, 33, 35, 37, 39, 40, 42, 43, 45–47
Cottidae	
Cottus gobio L. – bullhead	19,20, 25–27, 31, 32, 34, 36, 38, 40, 42, 43
Centrarchidae	
Lepomis gibbosus (L.) – pumpkinseed	3–10, 15, 18, 20, 22, 46
Percidae	
Perca fluviatilis L. – perch	1, 3–12, 15, 18–20, 22–24, 28, 31, 34, 35, 45, 46

species ranged from 1 to 17, with an average of 7.7. The diversity indices varied between 0 and 2.08 for H and 0.11 and 2.27 for I, respectively. The ABC index ranged from −16.48 to 11.41 and the IBI from 1.22 to 4.63. Sites 20–63, containing low metal loads, always had IBI values of more than 3.00.

3.3. Environmental and tissue toxic units

Toxic units based on the metal levels in the water and the water quality criteria (TU_w), ranged from 0.07 to 11.8 (Table 5). At sites 20–47, TU_w was always ≤ 1. Tissue toxic units (TU_t) were only measured in fish from

Table 5
Calculated indices of fish community structure at the different sampling sites, along with calculated toxic units

Site	N	H	I	ABC	IBI	TU_w	TU_t
1	10	1.66	0.54	1.55	3.38	1.55	7.95
2	3	0.92	0.90	3.18	1.22	11.8	38.5
3	6	1.47	0.93	−6.70	1.75	7.89	23.7
4	7	1.38	0.94	−4.22	2.25	10.8	32.0
5	13	1.90	0.91	0.83	2.88	4.01	6.60
6	17	2.08	0.72	−3.18	3.63	2.22	2.68
7	13	2.03	1.30	6.32	3.38	1.33	4.42
8	11	1.98	1.05	3.75	3.38	0.77	2.13
9	11	1.41	1.05	6.72	3.29	0.57	1.14
10	12	1.52	1.13	−5.29	3.25	0.59	1.90
11	11	1.84	1.51	−1.01	3.25	0.98	1.01
12	11	1.35	1.17	−2.54	3.25	0.63	ND
13	4	0.52	0.64	1.19	3.00	1.26	6.65
14	2	0.54	0.55	NC	3.13	0.87	10.6
15	6	1.75	2.27	23.9	2.75	0.73	12.5
16	2	0.29	0.58	NC	2.75	1.97	14.9
17	1	0.00	0.32	NC	2.25	1.34	20.1
18	12	1.31	0.52	−7.96	3.38	0.28	1.22
19	9	1.19	0.63	−1.72	4.25	0.12	1.16
20	9	1.73	0.47	4.02	3.75	0.28	1.00
21	6	1.26	0.33	5.53	4.25	0.61	1.17
22	11	1.93	0.93	9.12	3.50	0.18	3.87
23	11	1.80	1.66	6.20	3.38	0.46	3.49
24	10	1.12	0.41	2.54	4.00	1.25	ND
25	3	0.13	0.17	−10.4	4.00	0.34	ND
26	7	1.40	1.04	6.49	4.00	0.11	ND
27	3	0.10	0.18	−0.97	3.62	0.09	ND
28	7	1.41	0.35	8.39	4.00	0.11	ND
29	4	0.68	0.31	−12.6	3.62	0.08	ND
30	9	1.21	0.25	−5.29	4.62	0.11	ND
31	4	1.08	0.53	−16.5	3.62	0.08	ND
32	3	0.59	0.12	−8.82	3.88	0.09	ND
33	5	1.18	0.31	8.17	4.00	0.18	ND
34	8	1.10	0.72	−1.22	3.75	0.10	ND
35	5	0.26	0.28	0.45	3.62	0.13	ND
36	2	0.59	0.14	−0.95	3.62	0.26	ND
37	6	0.83	0.31	−13.0	3.12	0.09	ND
38	3	0.22	0.14	−8.51	3.75	0.11	ND
39	4	0.27	0.19	1.11	3.88	0.09	ND
40	4	0.86	0.55	−5.15	3.12	0.08	ND
41	1	0.00	0.11	NC	4.00	0.07	ND
42	6	1.41	0.41	11.0	3.88	0.14	ND
43	4	0.77	0.35	−6.71	3.38	0.17	ND
44	6	1.02	0.41	−7.11	3.00	0.15	ND
45	8	1.33	0.61	0.20	3.12	0.11	ND
46	12	1.34	0.91	−2.16	3.50	0.12	ND
47	6	1.13	0.68	4.96	3.88	0.10	ND

N, number of species; H, diversity index of Shannon; I, diversity index of Mehinck; ABC, abundance biomass comparison index; IBI, index of biotic integrity; NC, not calculated; ND, not determined; TU_w, toxic units based on water quality standards; TU_t, tissue toxic units based on background levels in liver of gudgeon.

sites 1–23. At these sites TU_t varied between 1 and 38.5 (Table 5).

If all sites were considered, a significant positive correlation was observed between the two different TU approaches (Table 6). Furthermore, TU_t and TU_w were significantly negatively correlated with IBI, and TU_t also with the number of species (N). No significant correlations were observed between TU_t or TU_w and the ABC or diversity indices H and I. The ABC index and N correlated significantly with H and I (Table 6). A significant correlation was also observed between both diversity indices.

Fig. 2 shows the linear relationship between the TU_w and IBI. If all sites were considered, 56% of the variation in the observed IBI was described (Fig. 2a). Considering all the metal-polluted sites (sites 1–23), 63% of the variation was described (Fig. 2b). When only the metal pollution gradient was considered (sites 1–12), 85% of the variation was described (Fig. 2c).

Relating the TU_t to the IBI at all metal polluted sites (sites 1–23) could describe 81% of the observed variation in IBI (Fig. 3a). Considering only the pollution gradient (sites 1–12), 87% of the variation was described (Fig. 3b).

If only sites 1–7 were considered, all belonging to the same river with very comparable river characteristics (depth, width, current speed), a very good correlation ($r^2=0.91$) was found between TU_t and the Shannon diversity index. Including the sites more downstream (8–12), the described variation decreased to 57%.

4. Discussion

In the present study, different relationships were observed between estimates of metal contamination and fish community structure in Flemish watercourses. However, considering all sampled sites no significant correlations were found between toxic units (TU_t, TU_w) and the diversity indices of Shannon (H) and Menhinick (I). The use of diversity indices is based upon the concept that changes in community structure are proportional to the degree of perturbations in the environment (Hellawell, 1989). However, no assumptions are made regarding the nature of the stress. As a consequence, fish diversity may not only be related to (metal) pollution but also to other factors like food availability or physical (river dimensions, river discharge, presence of woody debris and or aquatic vegetation, etc.) and/or chemical (pH, water hardness, oxygen level, etc.) characteristics of watercourses (Hellawell, 1989; Metcalfe, 1989; Matthews, 1998; Thiebaut et al., 2002; De Haas, 2004). This was obvious when TU_t was related to the fish diversity if only sites 1–7 were considered. At these sites all river and water characteristics but metal levels were comparable. For those sites, 91% of the variation in the Shannon index was described by the TU_t. When the other sites of the pollution gradient in the Molse Nete–Grote Nete river system were included in the analysis, the described variation decreased to 57%. This is probably due to the increased dimensions of the river and changes in some water characteristics,

Table 6
Pearson correlation matrix of the different indices and toxic units

	TU_w	TU_t	ABC	*N*	*H*	*I*	IBI
TU_w	1						
TU_t	0.980^{***}	1					
ABC	-0.027^{ns}	-0.193^{ns}	1				
N	-0.097^{ns}	-0.601^{*}	0.153^{ns}	1			
H	0.113^{ns}	-0.360^{ns}	0.483^{**}	0.430^{**}	1		
I	0.227^{ns}	0.021^{ns}	0.511^{**}	0.460^{**}	0.634^{**}	1	
IBI	-0757^{***}	-0.870^{***}	0.033^{ns}	-0.072^{ns}	-0.093^{ns}	-0.438^{**}	1

R-values are reported. *N*, number of species; *H*, diversity index of Shannon; *I*, diversity index of Mehinck; ABC, abundance biomass comparison index; IBI, index of biotic integrity; TU_w, toxic units based on water quality standards; TU_t, tissue toxic units based on background levels in liver of gudgeon.
* $p<0.05$
** $p<0.01$; ns, not significant.

which besides metal pollution also affect the diversity index. When all sites were included, no significant relationship was found any longer. In agreement with our results, Sawyer et al. (2004), considering 48 sites in the Choctahatchee-Pea watershed in Alabama, found only poor or no significant correlations between fish diversity indices and water quality characteristics. Within one river basin, however, Mol and Ouboter (2004) showed a decreased fish diversity when applying the Shannon index, downstream from a gold mine.

No significant relationships were found between the ABC-index and the TU_w or TU_t, even when only the pollution gradient in the Molse Nete–Grote Nete river system was considered. To the best of our knowledge, only two studies so far used the ABC-index to assess the effect of stress on freshwater fish populations. Coeck et al. (1993) tested the ABC-method to evaluate the effect of river regulation on fish communities. Nine sites of good water quality, with comparable dimensions (i.e. depth and width) but differing habitat quality were compared in that study. Moreover, within one river 10 trajects with the same water quality but differing physical habitat quality were compared. The ABC-index varied from −11.5 to 9.74. A good correlation was found between the ABC index and the habitat quality. Penczack and Kruk (1999) did not find a consistent relationship between pollution and the ABC-index when applied on fish communities at 48 sites in the Pilica river, Poland. Both studies indicate that other factors besides water quality are important in the determination of the ABC-index. In our study the selected sites contained river trajects with different degrees of habitat degradation. This might be a reason for the absence of any relationship with water pollution. Nevertheless, although all selected sites within the pollution gradient were comparable in their poor habitat quality, no relationship between ABC and TUs was found. The applicability of the ABC method, using fish populations for the assessment of water pollution, has not yet been demonstrated. In undisturbed first order watercourses, it is likely to find mainly small fish species, such as the bullhead (*Cottus gobio*) and stone loach (*Barbatulla barbatulla*). Such species are rather sedentary and can be locally present in relatively large numbers (Bruyndoncx et al., 2002; Knaepkens, 2004). As a consequence, although the environmental stress is low, this might result in a negative ABC-index (e.g. sites 25, 31, 32). Moreover, many of the sensitive fish species are small (e.g. bullhead, spined loach, brook lamprey), whereas a lot of pollutant resistant species are rather large (roach, bream, eel; Belpaire et al., 2000).

The IBI was the only index that was significantly correlated with toxic units. Two important observations, however, have to be made concerning this relationship. First, when relating the TU_w to the IBI, the described variation ranged from 56% to 85% depending on which sampling sites were included in the analysis. Apparently other factors besides water pollution had an effect on the IBI. From Fig. 2a it was clear that above a threshold TU_w of 4 the IBI was never higher than 3.00. This indicates that metal pollution indeed is reflected in the IBI. Nevertheless, below this threshold, the IBI fluctuated between 2.25 and 4.63. Since all the corresponding sites were low in metal pollution and had good water quality characteristics, the variation in the IBI has to be influenced by other factors such as habitat quality. These results are consistent with the findings of other studies. For example Hartwell et al. (1997), who correlated ambient toxicity with fish community structure at tributaries of Chesapeake Bay (USA), found significant relationships between the toxicological risk of a site and the IBI, although effects of habitat deficiencies were also found. Likewise, Dyer et al. (2000b) not only found significant negative correlations between toxic units and IBI in the basin of the Great Miami River (USA), but also found important effects of habitat quality.

Second, within the metal polluted sites, the described variation increased from 63% to 81% if the IBI was related to the TU_t instead of the TU_w. If only the pollution gradient was considered, the described variation further increased to 87%. These results illustrate

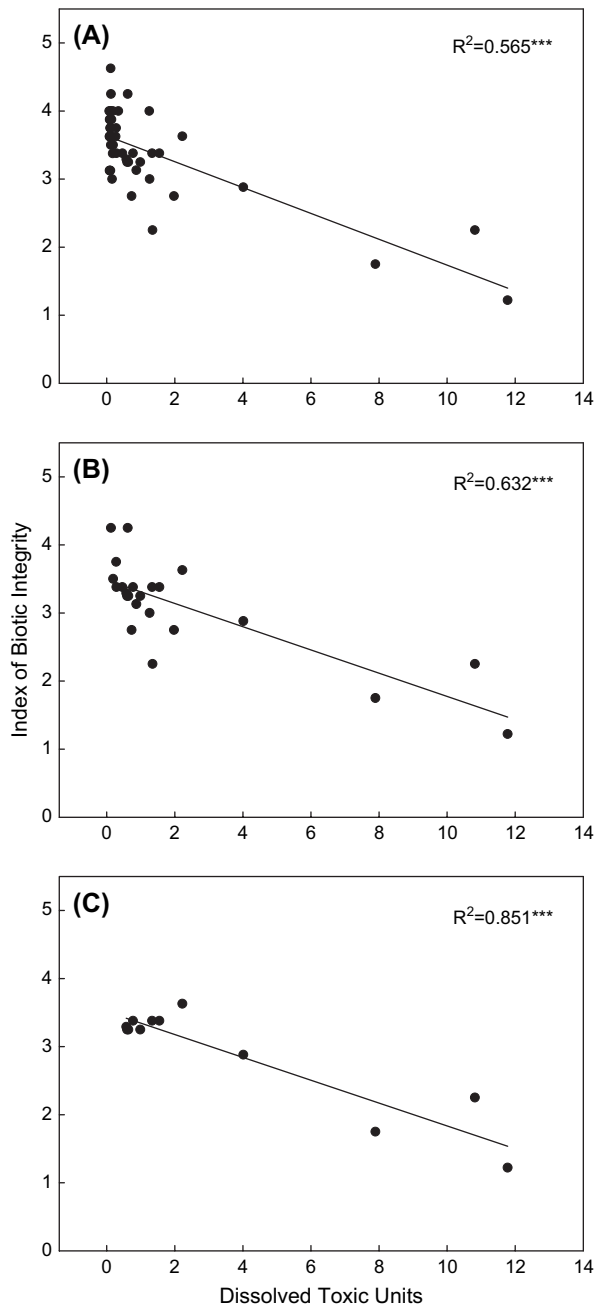

Fig. 2. Index of biotic integrity versus dissolved toxic units based on metal levels in the water. (A) All sampling sites; (B) sites 1–23, metal polluted sites; (C) sites 1–12, metal pollution gradient.

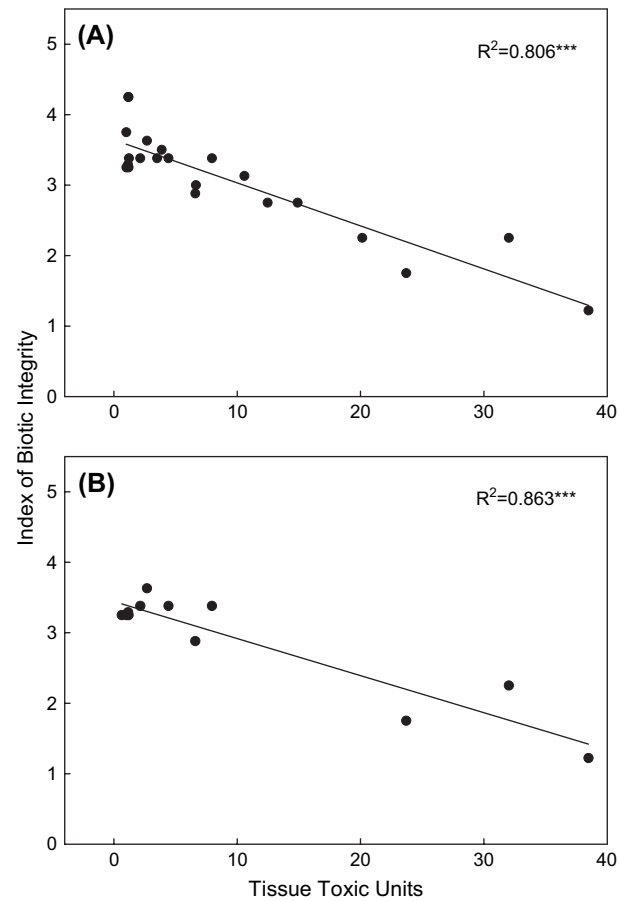

Fig. 3. Index of biotic integrity versus tissue toxic units, based on metal levels in liver of gudgeon. (A) Sites 1–23, metal polluted sites; (B) sites 1–12, metal pollution gradient.

that it is also important to include the bioavailability of the metals in the analysis. At some sites, environmental metal levels might be high although accumulation is lower than expected due to metal complexation (Hare and Tessier, 1996). On the other hand, dissolved metal levels can be low but metals might be accumulated in the sediment and invertebrates (Luoma, 1989).

The results of the current study clearly demonstrated that even at very high environmental and/or tissue metal levels, a considerable number of fish species were still present (e.g. sites 3 and 4). On the other hand, some sites with low levels of metals and good water quality characteristics contained very few species (e.g. sites 27, 36, and 41). Since fish are relatively mobile, it is very likely that only resistant life stages or species will be present at the contaminated sites. This of course is not reflected in diversity indices or the ABC-index. Since the IBI combines a set of metrics, including species sensitivity and recruitment, effects on the most sensitive species and life stages will be reflected in the index. In general, we can conclude from this study that metal pollution in aquatic ecosystems may be assessed by fish community structure. The IBI proved a suitable index for the assessment of metal pollution, provided that other water quality characteristics meet the water quality criteria and are not negatively influencing the fish community structure.

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