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Use of a macroinvertebrate based biotic index to estimate critical metal concentrations for a good ecological water quality.

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Abstract

Large datasets from total and dissolved metal concentrations in Flemish (Belgium) fresh water systems and the associated macroinvertebrate-based biotic index MMIF (Multimetric Macroinvertebrate Index Flanders) were used to estimate critical metal concentrations for a good ecological water quality, as imposed by the European Water Framework Directive (2000). The contribution of different stressors (metals and water characteristics) to the MMIF were studied by constructing generalized linear mixed effect models. Comparison between estimated critical concentrations and the European and Flemish EQS, shows that the EQS for As, Cd, Cu and Zn seem to be sufficient to reach a good ecological quality status as expressed by the invertebrate-based biotic index. In contrast, the EQS for Cr, Hg and Pb are higher than the estimated critical concentrations, which suggests that when environmental concentrations are at the same level as the EQS a good quality status might not be reached. The construction of mixed models that included metal concentrations in their structure did not lead to a significant outcome. However, mixed models showed the primary importance of water characteristics (oxygen level, temperature, ammonium concentration and conductivity) for the MMIF.

Keywords

Metals; Ecological water quality; Macroinvertebrates; MMIF; Quantile regression, Ecological Quality Standards; Belgium

Introduction

The ecological quality of an aquatic system is impacted by anthropogenic pressures, such as pollution, habitat deterioration, spatial isolation, climate change and the spreading of invasive species. All these pressures will affect the biological community in the aquatic environment. Since well-balanced and adaptive communities can only be maintained by a healthy aquatic ecosystem, the community structure will reflect the ecosystem's health. So, in order to assess the ecological status of a water body, biotic indices use the presence and abundance of aquatic species as a measure for the ecosystem's health. In 1964, Woodiwiss developed the Trent Biotic Index (TBI), a scoring system for water quality assessment in the UK based on macroinvertebrates (Woodiwiss, 1964). Since then, macroinvertebrate based biotic indices have been widely used and adapted to local conditions (Moya et al., 2011; Pond et al., 2013). In Belgium, De Pauw and Vanhooren developed a method based on the TBI and the French Biotic Index of Tuffery and Vernaux (1968).

In order to protect the aquatic environment from detrimental effects of contaminants, the European Union implemented the Water Framework Directive (WFD) in 2000. The WFD imposes a 'good quality status' for all European water bodies by 2015 (EU Water Framework Directive, 2000). Next to a good chemical status, also a good ecological status must be achieved. For the assessment of the ecological water status, monitoring should be based on the status of various biological quality elements including macroinvertebrates, phytoplankton, macrophytes, phytobenthos and fish fauna. For the assessment based on macroinvertebrates, several countries developed a multimetric biotic index, e.g. France (I2M2, Mondy et al., 2012), Sweden (Rosenberg et al., 2004), Switzerland (Menetrey et al., 2011), Italy (Solimini et al., 2008) and Portugal (Teixeira et al., 2009). In Flanders (northern part of Belgium), the Belgian Biotic Index (BBI, De Pauw and Vanhooren, 1983) was updated to the Multimetric Macroinvertebrate Index Flanders (MMIF, Gabriels et al., 2010) to comply with the WFD guidelines.

Knowing which environmental levels of pollutants are critical to reach a good ecological status, as imposed by the WFD, would provide governments the ability to formulate more accurate environmental quality standards (EQS) to protect the environment. Because of this, several studies have tried to link field data on pollution concentrations to ecological quality data (Crane et al., 2007; Iwasaki et al., 2011; Iwasaki and Ormerod, 2012; Stockdale et al., 2010).

In Flanders, industrial and domestic activities have caused the introduction of metals in the environment. In particular, the activities of the metallurgic industry in the nineteenth and twentieth century led to elevated metal concentrations into the aquatic environment (De Jonge et al., 2008; Groenendijk et al., 1999). With a broad range of environmental metal concentrations, Flanders is an interesting area to explore the relationships between environmental metal concentrations and ecological water quality.

The aim of this study was to investigate whether critical metal concentrations for a good ecological water status could be estimated from environmental data and whether current EQS are protective enough to reach a good status. To do this, large datasets on water metal concentrations and MMIF scores from 1189 individual sites in Flanders were combined. In addition, the contribution of different water characteristics including oxygen content, water conductivity, ammonium level and pH to the MMIF is determined.

Material and Methods

2.1 Sampling

Field data were retrieved from a monitoring program of the Flemish Environment Agency (VMM). Water metal concentrations were measured with ICP-MS (Inductively Couple Plasma- Mass Spectrometry, Agilent 7500). For dissolved metal concentrations, water samples were first filtered over 0.45 µm membrane filters (Whatman Polydisc GW Filter). Measurements below limits of quantification (LOQ) were treated as half the LOQ value of the compound considered. In addition to the metal concentrations, water characteristics were measured on each location. Oxygen level, oxygen saturation, temperature, pH and conductivity were determined *in situ* with a digital multi meter (Hach-Lange). The ammonium concentration in the water samples was measured in the laboratory with an ammonium analyser (Skalar).

For the ecological quality data, only assessments performed within 90 days after the sampling of the water for metal analyses, were included in the present study. Individual metal concentrations were linked with the MMIF from the same location. Combination of both databases led to paired data from 10118 measurements at 1189 sampling locations. Table 1 gives an overview of the number of samples and sites per metal. Macroinvertebrates were collected as described by De Pauw en Vanhooren (1983) and Gabriels et al. (2010). With a standard handnet (200 x 300 mm frame, 300-500 µm mesh), a stretch of 10-20 m river was sampled for 3 to 5 minutes. All accessible aquatic habitats were equally sampled. Next to kicksampling with the handnet, animals were picked up manually to ensure collection of the highest possible diversity of macroinvertebrates from all habitats. At locations were the water was too deep to perform kicksampling, macroinvertebrates were collected using artificial substrates (De

Pauw et al., 1986). The substrates were placed along the banks of the water bodies and recollected after at least three weeks of incubation. All collected macroinvertebrates were identified according to the taxonomic levels described by De Pauw en Vanhooren (1983). The MMIF was calculated according to Gabriels et al. (2010). Metrics determined for the calculation of the MMIF were: taxa richness, number of Ephemeroptera, Plecoptera and Trichoptera taxa (EPT), number of other (non-EPT) sensitive taxa, the Shannon-Wiener Diversity (SWD; Shannon and Weaver, 1949) index and the mean tolerance score. The MMIF calculations resulted in a score ranging from 0 to 1, with score 0 representing locations with a bad biological quality and score 1 representing high ecological conditions.

2.2 Statistical analyses

Statistical analyses were performed using R 2.15.2 (R Development Core Team, 2011) and GraphPad Prism 5.04 (GraphPad Software, Inc). To estimate the critical metal concentrations, two approaches were used. The first approach was the use of quantile regression. This regression technique is often used when an ecological response can be affected by several environmental factors next to the stressors included in the study (e.g. habitat, species interactions, other pollutants,…) (Crane et al., 2007; De Jonge et al., 2013; Iwasaki and Ormerod, 2012; Linton et al., 2007; Schmidt et al., 2011). In this case, the maximum MMIF score at a certain location can be limited by the presence of metals, while at another location with the same metal load, another influencing factor may lead to a lower MMIF score. By considering the 90th percentile of all MMIF scores, only the maximum ecological response is taken into account, to compensate for these unmodelled factors. The 90th quantile regression models ($\tau = 0.9$) were constructed using the quantreg package of the statistical software R (Koenker, 2005). Some outliers were removed from the dataset after screening on QQ plots. They were all datapoints with high metal concentrations and low MMIF scores. Based on the significant constructed regression models critical metal concentrations representing a certain percentage of decrease in MMIF could be calculated. For the second approach, concentration thresholds were established as the 95th and the 99th percentile of the metal concentrations measured at locations were a good quality status was achieved. For a good status, the MMIF score needs to be higher than 0.6 for polder watercourses and higher than 0.70 for other water bodies (Gabriels et al., 2010). These statistical approaches were performed on total and dissolved metal concentrations.

To investigate the contribution of the metals and water characteristics to the MMIF, generalized linear mixed effect models were constructed (Zuur et al., 2009). Dissolved metal concentrations and water characteristics were set as fixed variables in the model while the categorical variables date and sampling location were set as random variables, in order to control for pseudo replication. Determination of the most parsimonious model was performed using Akaike's Information Criterion (AIC, Johnson and Omland, 2004). The optimal random structure was selected using restricted maximum likelihood, prior to model selection of the most parsimonious fixed effect structure. After model selection, the most parsimonious model was refitted using the full dataset. An R^2 was calculated by squaring the correlation coefficient of the correlation between the fitted model values and the actual observations.

Results and discussion

3.1 Description of the database

The combination of the metal concentrations in the water and the MMIF scores at the same locations led to an unbalanced dataset, both temporally and geographically. In the combined dataset, a higher number of measurements per year are included from the period 2000-2006, compared to the period 2007-2011. On average, each location was sampled 8.5 times, but the number of repeated measurements ranged from 1 to 53. Table 2 presents the mean, median and ranges of the total and dissolved metal concentrations, MMIF scores and water characteristics used in this study. Average dissolved metal concentrations did not exceed Flemish (VLAREM II, 2010) or European (EU, 2008) directives as shown in Table 5. Nonetheless, for every metal, some samples exceeded the EQS, ranging from 1.2% of the samples for Cr to 32.6% for Zn. The median MMIF score was 0.40. Only 1301 out of 10118 quality assessments (12.9 %) resulted in a MMIF score representative of a good quality status. MMIF scores were positively correlated with oxygen content (Pearson $r = 0.342$, $p < 0.0001$, $n = 9953$) and oxygen saturation (Pearson $r = 0.359$, $p < 0.0001$, $n = 9959$) and negatively correlated with ammonium (Spearman $r = -0.627$, $p < 0.0001$, $n = 8550$).

3.2 Critical metal concentrations

Figure 1 and 2 represent the scatterplots for the MMIF versus total and dissolved metal concentrations respectively, plotted on a logarithmic scale. Significant quantile regression models ($\tau = 0.9$) for the change in maximum MMIF in function of total water concentrations, applying all data, could be constructed for Cr only. After removing outliers from the dataset, significant models could also be constructed for As, Cu and

Hg. For Cd, Ni, Pb and Zn no significant models could be obtained. The significant 90^{th} regression quantiles and equations are shown in Figure 1. Metal concentrations corresponding with a decrease in MMIF of 5, 10 and 20% are represented in Table 3, together with the concentration thresholds based on the 95^{th} and the 99^{th} percentile of the total metal concentrations at locations with a good quality status.

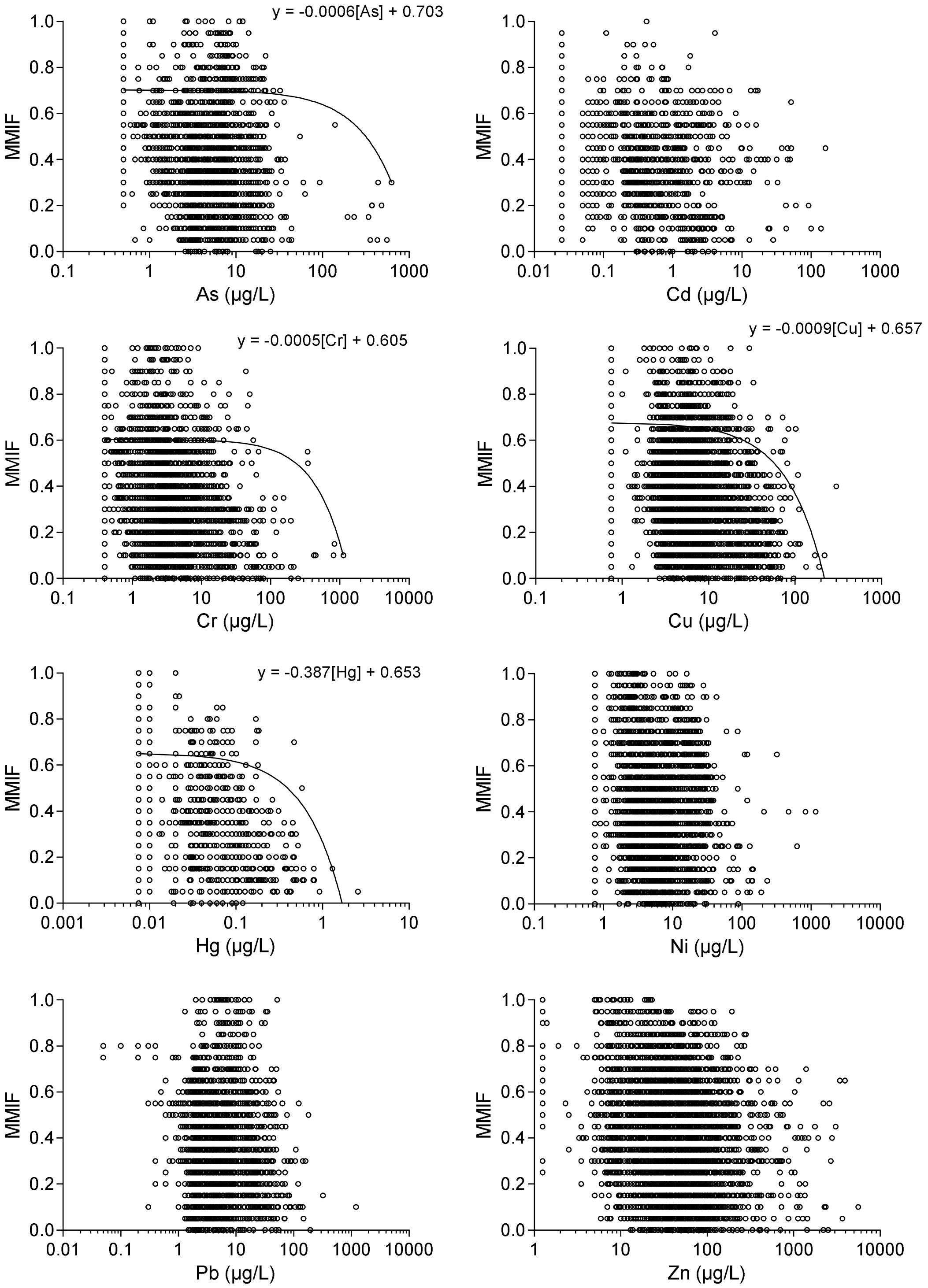

Figure 1. Scatterplots of MMIF in relation to total metal concentration for all metals, together with the 90th quantile regression models for the change in maximum MMIF as

function of total water concentrations of As, Cu, Cr and Hg. The X-axis is displayed on a logarithmic scale.

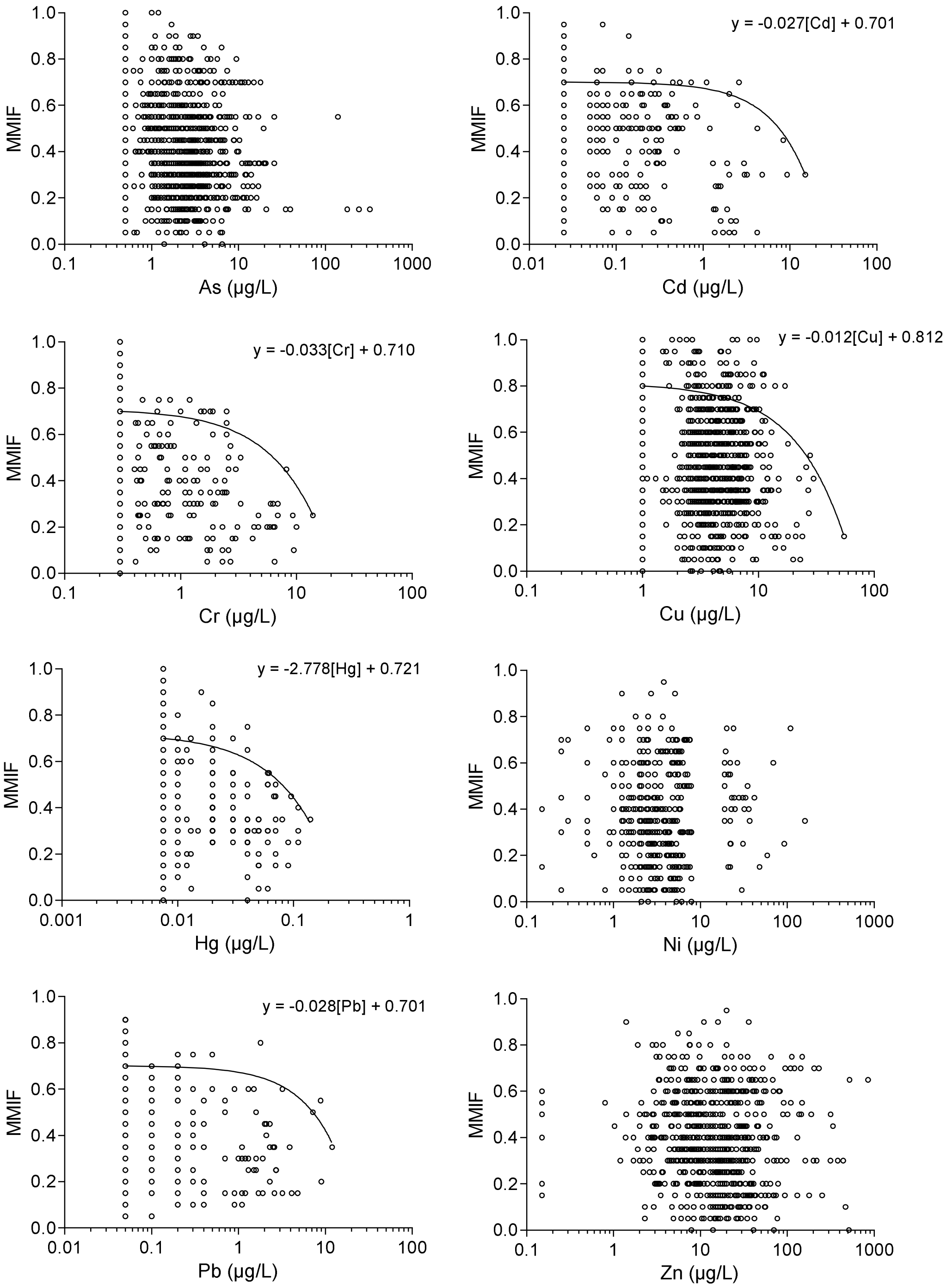

Figure 2. Scatterplots of MMIF in relation to dissolved metal concentration for all

metals, together with the 90th quantile regression models for the change in maximum MMIF in function of dissolved water concentrations of Cd, Cr, Cu, Hg and Pb. The X-axis is displayed on a logarithmic scale.

For Cu and Hg, the 95th percentile of the total metal concentrations at locations with a good ecological status, is within the concentration ranges corresponding to a 5 or 10% loss in MMIF, calculated by quantile regression. In the case of As and Cr, the 95th percentile value is much lower than the concentration corresponding to a 5% loss in MMIF.

For the dissolved metal concentrations, significant quantile regression models ($\tau = 0.9$) for the change in maximum MMIF, applying all data, could be constructed for Cr and Hg. After removing outliers from the database, significant regression models could be constructed for Cd, Cu and Pb. The significant 90th regression quantiles for the dissolved metal concentrations are presented in Figure 2. Dissolved metal concentrations corresponding with a decrease in MMIF of 5, 10 and 20% are presented in Table 4.

Crane et al (2007) performed a comparable study on field data from England and Wales. Dissolved metal concentrations were related to a decrease in EQI score (ecological quality index), number of Ephemeroptera families and number of EPT families. Suggested ranges for the standards for dissolved metals are also presented in Table 4. In general, the critical concentrations in our study are higher, except for Cr and Pb. The two percentile values of Cr and Pb and the concentrations corresponding to a 5% and a 10% loss in MMIF are comparable to the results of Crane et al (2007). Iwasaki and Ormerod (2012) estimated safe concentrations for dissolved metals by relating them to a standardized measure of EPT richness from locations in the UK, USA and Japan. In the present study, higher critical concentrations were found for Cd (Table 4). The values for Cu and Zn, fit well in the 95% confidence intervals of Iwasaki and Ormerod.

Next to the estimations of the critical metal concentrations from the present study, Table 4 shows the official EQS from Flemish (VLAREM II, 2010) and European (EU, 2008) legislation. Comparison shows that for As, Cd and Zn, the EQS is well below the estimated critical concentrations, so compliance with the EQS would be sufficient to reach a good ecological quality status. The EQS for Cu lies below the two percentile values of the concentrations at locations with a good status and is comparable with the concentration corresponding with a 10% loss in MMIF. In contrast, the EQS of Cr and Pb are higher than the estimated critical concentrations. This results suggest that when environmental concentrations are at the same level as the EQS, a loss in MMIF can occur and a good quality status will not be reached. As mentioned before, our estimated concentrations for Cr and Pb are comparable to the results from Crane et al (2007). Similarly, the EQS for Hg is higher than the two percentile values and is comparable with the concentration corresponding with a 20% MMIF loss. The EQS for Ni lies within the 95th and the 99th percentiles of the concentrations at locations with a good status.

It is important to note that these scatterplots and statistical approaches do not prove a causal relationship between the metal concentrations and the presence or absence of a good ecological quality. Furthermore, site dependent differences in the bioavailability of the metals is not explicitly considered in this analysis. There are many direct and indirect interactions between the biotic and abiotic factors in the field situation. Nonetheless, since these large datasets include many different field situations in Flanders, the critical concentrations formulated in this study will have a predictive value, at least for field situations in Flanders, but most likely also for other aquatic systems. Moreover, current EQS also do not consider bioavailability. Our datasets are adequate to test their efficiency in protecting the aquatic community.

Table 5 compares the most conservative range of critical concentrations from the three studies discussed above with current water quality guidelines from Flanders (Europe) (VLAREM II, 2010; EU, 2008), Australia (ANZECC, 2000), Canada (CCME, 2003) and the US (US EPA, 2001). Comparison shows that for As, the Flemish guideline is well below the estimated critical concentrations. However, the guideline for Cu, Cr, Hg, Ni and Pb in Flanders, is higher than the lowest estimated critical concentrations. For Zn, the Flemish guideline is within the range suggested by Iwasaki and Ormerod (2012), and lower than the critical concentrations in the other two studies. This indicates that the guideline will be protective enough. Australian guidelines (99%) seem sufficient for concentrations of As, Cd, Cr, Zn, but the guidelines for Cu, Hg and Ni might lead to a loss in ecological quality. Comparison with Canadian guidelines show that for As, Cd and Hg, the guidelines will assure a good ecological water status. For Cr however, this may not be sufficient. US guidelines appear not protective enough for concentrations of As, Cr, Hg, Ni and Pb. The guidelines for Cd and Zn are within the lowest critical concentration range.

3.3 Factors influencing the MMIF

Concentrations of Ni were excluded from the mixed models, because of the high correlation with Zn (Pearson correlation; $r=0.71$, $p<0.0001$). According to AIC, the most parsimonious model only contained sampling location as random structure. In the full model, five water characteristics and the dissolved concentrations of the seven metals were included. For this model structure however, no significant model could be constructed. Furthermore, when only the seven metals were included as fixed structures, no significant model was found. When only the water characteristics were included in the full model, the most parsimonious model resulted in the following equation: $MMIF = -0.554 + (0.063*O_2) + (0.027*T) + (-0.0005*EC20) + (-0.087*NH_4)$. pH was not retained in the model. The other four variables all made a significant contribution to the model. The correlation between the fitted model and the observed data showed that 27% of the total variation was explained by the model ($R^2 = 0.2667$ with $N = 3566$). The results of this mixed model suggest that the water characteristics are of primary importance for the structure of the macroinvertebrate community. Nonetheless, only 27% of the variation is explained, meaning that there are many other significant variables not included in the model.

In conclusion, the construction of mixed models in this study showed the primary importance of water characteristics for the MMIF. Models that included metal concentrations in their structure did not lead to a significant outcome. However, by means of formulating 95th and 99th percentiles of the concentrations at locations with a good status and using 90th quantile regression models, critical concentrations could be estimated above which a good status is unlikely to be reached.

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Table 1. Number of sampling locations and samples per metal

	Total concentration		Dissolved concentration	
	Sampling locations	Samples	Sampling locations	Samples
Arsenic	660	2216	396	1100
Copper	968	5044	447	1514
Cadmium	473	1610	255	733
Chromium	681	3360	409	1197
Mercury	427	1925	393	1452
Nickel	784	4871	233	725
Lead	735	3194	138	318
Zinc	1144	8202	307	818

Table 2. Mean, median and range of water chemistry and MMIF scores at all sampling locations

	Unit	Mean	Median	Minimum	Maximum
Total concentrations					
As	µg/L	14.6	5.50	<1.00	4980
Cu	µg/L	10.0	6.20	<1.25	1570
Cd	µg/L	1.64	0.30	<0.05	162
Cr	µg/L	7.74	3.00	<0.80	1130
Hg	µg/L	0.05	0.03	<0.015	5.70
Ni	µg/L	9.51	6.20	<1.50	1170
Pb	µg/L	9.59	6.00	<0.10	1210
Zn	µg/L	56.6	30.0	<2.50	5580
Dissolved concentrations					
As	µg/L	3.78	2.00	<1.00	325
Cu	µg/L	3.60	2.60	<1.25	265
Cd	µg/L	0.43	0.03	<0.05	47.3
Cr	µg/L	0.52	0.30	<0.60	13.9
Hg	µg/L	0.01	0.01	<0.015	0.14
Ni	µg/L	4.56	2.50	0.15	160
Pb	µg/L	0.58	0.05	<0.10	22.0
Zn	µg/L	26.4	14.0	<0.3	849
MMIF	-	0.41	0.40	0.00	1.00
O2	mg/L	6.89	6.70	0.10	29.9
O2 sat.	%	68.1	67.0	1.00	348
T	°C	15.2	15.8	0.00	30.0
EC 20	µS/cm	613	617	1.00	2072
pH	-	7.64	7.70	4.10	10.7
NH4+	mgN/L	2.75	1.30	0.04	200

Table 3. Critical total metal concentrations (µg/L) corresponding to 5, 10 or 20% loss in MMIF based on significant 90th quantile regression models (left) and calculated as the 95th and 99th percentile of the concentrations at locations with a good status (right). n.s.: no significant regression model. For As, Cu and Hg regression models were only significant after removing outliers from the dataset.

	% Loss in MMIF			Percentile	
	5	10	20	95	99
As	54.9	110	220	19.0	22.0
Cu	10.9	21.9	43.8	15.0	24.4
Cd	n.s.	n.s.	n.s.	3.77	15.2
Cr	67.2	134	269	10.8	29.9
Hg	0.08	0.17	0.34	0.09	0.18
Ni	n.s.	n.s.	n.s.	21.0	31.0
Pb	n.s.	n.s.	n.s.	20.0	40.0
Zn	n.s.	n.s.	n.s.	124	269

n.s.: no significant regression model

Table 4. Critical dissolved metal concentrations (µg/L) corresponding to 5, 10 or 20% loss in MMIF based on significant 90th quantile regression models and calculated as the 95th and 99th percentile of the concentrations at locations with a good status, together with the the Flemish[a] (VLAREM II, 2010) and European[b] (EU, 2008) EQS and the critical concentrations suggested by Crane et al (2007) and Iwasaki and Ormerod (2012).

µg/L	% Loss in MMIF			Percentile		EQS Flanders	Crane et al. (2007)	Iwasaki and Ormerod (2012)
	5	10	20	95	99			
As	n.s.	n.s.	n.s.	9.50	14.5	3.00[a]		
Cu	3.37	6.74	13.5	8.82	11.1	7.00[a]	0.2 – 0.5	6.6 (1.2-14.2)
Cd	1.31	2.62	5.25	0.44	1.39	0.08-0.25[b]	0.2 – 0.5	0.11 (0.06-0.49)
Cr	1.07	2.15	4.29	1.16	1.88	5.00[a]	1.0 – 2.0	
Hg	0,01	0.03	0.05	0.01	0.02	0.05[b]		
Ni	n.s.	n.s.	n.s.	13.2	65.7	20.0[b]	0.6 -7.0	
Pb	1.25	2.51	5.02	0.40	1.41	7.20[b]	0.6 – 2.5	
Zn	n.s.	n.s.	n.s.	145	216	20.0[a]	20 - 27	34 (11-307)

n.s.: no significant regression model

Table 5. Most conservative range of critical metal concentrations from 3 studies (present study[a], Crane et al., 2007[b] and Iwasaki and Ormerod, 2012 [c]) compared with current quality guidelines. All guidelines are for chronic exposure in freshwater.

µg/L	Lowest range	Flanders (Europe)	Australia[f] 95% level of protection (99%-80%)	Canada[g]	US[h]
As	9.50-14.5[a]	3.00[d]	AsIII 24.0(1.00-360); AsV 13.0(0.80-140)	5.00	150
Cu	0.20-0.50[b]	7.00[d]	1.40 (1.00-2.50)		*BLM
Cd	0.06-0.49[c]	0.08-0.25[e]	0.20 (0.06-0.80)	0.09	0.25
Cr	1.00-2.00[b]	5.00[d]	1.00 (0.01-6.00)	CrVI 1.00; CrIII 8.90	CrVI 11.0; CrIII 74.0
Hg	0.01-0.05[a]	0.05[e]	0.60 (0.06-5.40)	26.0 ng/L	0.77
Ni	0.60-7.00[b]	20.0[e]	11.0 (8.00-17.0)		52.0
Pb	0.40-1.41[a]	7.20[e]	3.40 (1.00-9.40)		2.50
Zn	11.0-307[c]	20.0[d]	8.00 (2.40-31.0)		120

[a] present study
[b] Crane et al. (2007)
[c] Iwasaki and Ormerod (2012)
[d] VLAREM II (2010)
[e] EU (2008)
[f] ANZECC (2000)
[g] CCME (2003)
[h] US EPA (2001)
*to be calculated with Biotic Ligand Model (US EPA, 2001)

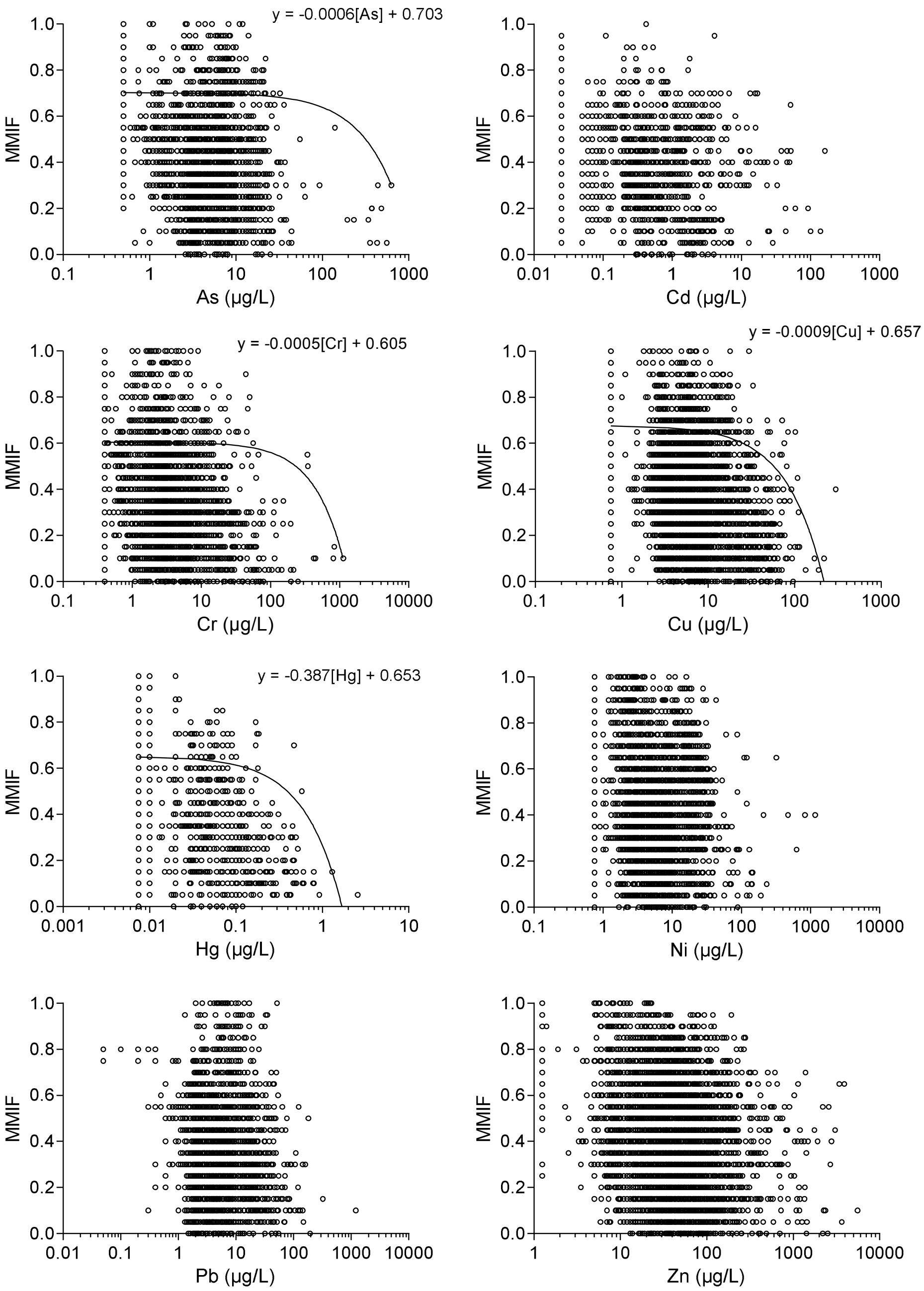

Figure 1. Scatterplots of MMIF in relation to total metal concentration for all metals, together with the 90th quantile regression models for the change in maximum MMIF in function of total water concentrations of As, Cu, Cr and Hg. The X-axis is displayed on a logarithmic scale.

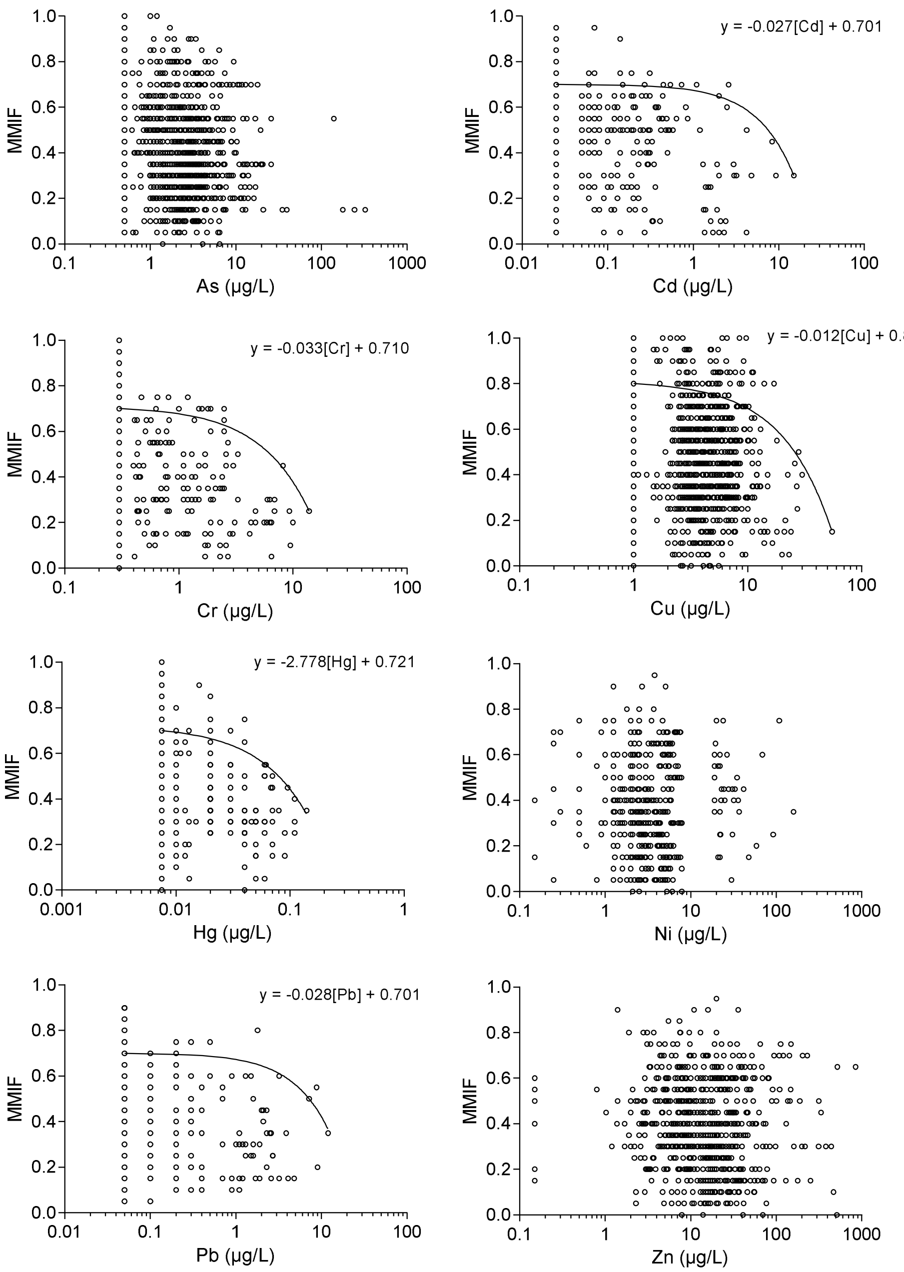

Figure 2. Scatterplots of MMIF in relation to dissolved metal concentration for all metals, together with the 90th quantile regression models for the change in maximum MMIF in function of dissolved water concentrations of Cd, Cr, Cu, Hg and Pb. The X-axis is displayed on a logarithmic scale.