

The response of macroinvertebrate community taxa and functional groups to pollution along a heavily impacted river in Central Europe (Bílina River, Czech Republic)

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Abstract: Macroinvertebrate communities were investigated along a gradient of heavy industrial and municipal pollution in the highland Bílina River (Czech Republic). Physico-chemical determinants and ions were monitored and community analysis performed focusing on taxonomic composition, ecological functioning (feeder and dweller guilds) and water quality metrics, including saprobity index, BMWP and diversity. Impacted sites differed significantly from reference and from recovered stretches. Chemical data revealed two main pollution factors, (1) a “salinity determinant”, described best by conductivity and SO_4^{2-} , and (2) an “organic pollution determinant”, represented best by O_2 concentrations and NO_2^- , all varying locally and temporally. Some metrics and taxa showed significant correlations to abiotic parameters. Functional communities showed a stronger relationship to the “organic pollution determinant”, suggesting that elevated organic pollution had a dominating influence on functional community metrics; though other variables may also have an influence in this multi-stress environment. On the other hand, there were indications that the taxonomic community was more influenced by ion concentrations (“salinity determinant”). The gradient from reference sites to polluted sites was weaker in the final sampling campaign. The results presented here can be used as a reference for assessing future changes in environmental impact from pollution, being finer and more detailed than assessment according to the EU’s WFD.

Key words: community analysis; functional community; taxonomic community; multiple pollution; multi-stress; macroinvertebrates; Central Europe; lower mountain river; EU-WFD

Introduction

Changes in water quality along a river’s course affect not only taxonomic composition but also the function of a community. Numerous studies have used macroinvertebrates in order to trace the sources and extent of pollution or to monitor the degradation or recovery of a stream. In the Western Palearctic and Nearctic bioregions, traits and tolerances of macroinvertebrate taxa are sufficiently well known to allow appropriate bioindication levels to be applied (Griffiths 1991; Orendt 1999; D’Surney et al. 2000; Timm et al. 2001; Statzner et al. 2005; Berenzen et al. 2005; Gupta & Sharma 2005; de Deckere et al. 2007). One of the most frequently used tools for measuring biological water quality is the index of saprobity, which reflects pollution by organic, decomposable wastewaters. This was developed >100 years ago (Kolkwitz & Marsson 1902), and later extended and refined by Zelinka & Marvan (1961), Sládeček (1973), Mauch et al. (1985) and others. Nowadays, there is increasing awareness that rivers suf-

fer from multiple stressors (Tockner et al. 2010), which may confound successful implementation of the European Union’s Water Framework Directive (EU-WFD; EC 2000). In addition to saprobity, chemical pollution and loss of habitat may also contribute to changes, or even a decline, in macroinvertebrate assemblages. This has already been observed in the Bílina and Elbe rivers (Adámek & Jurajda 2001; Adámek et al. 2010) and has to be taken into consideration when searching for the key causes of ecological degradation. In the St. Clair River in Canada, Griffiths (1991), among others, found that physical habitat characteristics and sediment contaminants explained different kinds of macroinvertebrate communities and their numbers. Moreover, the influence of pollution on the taxonomic community and on ecological function depends on its concentration, which may have different effects on different taxa. De Lange et al. (2004), in their studies on the Rhine-Meuse delta, found that moderate levels of contamination affected the structure, but not the productivity, of the benthic macroinvertebrate community. Thus, when a

water body is to be restored, it is in the interests of water managers to determine exactly which factors are responsible for the degradation in order to fulfil the requirements of the EU-WFD. The Bílina River, a tributary of the Elbe River in the North-West of the Czech Republic, is considered one of the most polluted running waters in the country. According to Jurajda et al. (2010), the river is at high risk of failing the requirements of the WFD. Our knowledge about the biota of the Bílina River Basin is, however, quite limited. Recently, Jurajda et al. (2010) released the results of a first survey of macroinvertebrate and fish assemblages at locations along the main channel and some small tributaries.

The aims of this study were to (1) investigate the macroinvertebrate communities of this heavily impacted river along the pollution gradient in the main channel, and (2) to elaborate the factors responsible for the response of the community to general pollution along the stream. An analysis of organochemical compounds will be treated separately in a following publication.

Study area and sample sites

The Bílina River (Fig. 1), a tributary of the Elbe River, is located in the North-West of the Czech Republic and has a catchment area of 1,070.9 km². It arises from a spring in the Krušné hory Mts (785 m a.s.l.) and, after 84.2 km, the river flows into the Elbe River at Ústí nad Labem (132 m a.s.l.). Its course is interrupted by two reservoirs (river km 72.2, km 66.8) and diversion piping (km 60.4–57.4). The river is considered as the most polluted stream in the Czech Republic, having received pollutants from heavy industry, brown coal mining, energy and chemical industries, and municipal wastewater treatment plants (WWTPs) for more than 100 years.

Samples were taken from eight locations along the river's course (Fig. 1, Table 1). We avoided following a topographical gradient along the river so as not to hinder interpretation of the impact of chemical compounds. All sampling sites were similar in terms of hydromorphological character and belong to the same potamal stream zone, while data on aquatic communities presented by Jurajda et al. (2010) show a relatively high morphological homogeneity of river zone. Morphological variation between the study sites was low and evaluation of morphological feature data did not show a significant gradient, indicating no major influence on community distribution that could mask the direct response of biota the direct response of the biota to water quality. Further, there was no significant difference in pH from neutral values (7.47 ± 0.26 SD) between sites, suggesting that this system is well buffered by the geochemical background or organic pollutants. Mobilisation of toxic heavy metals due to acidity, therefore, does not appear to play a major role in pollution of the Bílina River.

The highest upstream site (km 65.2) is located downstream of a reservoir. Between this site and that following (km 59.0), the river is diverted through piping for three kilometres. Since these two sites are located upstream of the first major inflow of wastewater, they are considered as 'reference' sites for sites situated downstream. These sites do not represent true reference sites, as required by the WFD,

Table 1. Sampling site and event characteristics.

Stream km	65.2	59.0	49.3	45.1	31.1	18.5	0.2	
Site name	Jirkov, downstream Kyjicka reservoir	Komořany	Litvínov	Most	Obrnice	Chotějovice	Velvety	Ústí n.L.
m a.s.l.	285	251	244	238	216	206	175	138
Coordinates	50°30'33.93"N, 13°29'22.53"E	50°31'47.43"N, 13°33'51.23"E	50°32'31.57"N, 13°37'3.09"E	50°30'55.46"N, 13°39'1.86"E	50°30'49.2"N, 13°41'54.53"E	50°36'23.66"N, 13°51'28.69"E	50°36'21.81"N, 13°53'N39.26"E	50°39'23.39"N, 14°2'13°78'E
Pollution*	unpolluted	unpolluted	polluted	polluted	polluted	polluted	polluted	polluted
Sampling date	19–22 June 2006 24–27 Sep. 2007 22–24 Oct. 2007 22–24 Apr. 2008	+	+	+	+	+	+	+

Explanations: + sampling performed, – no sampling, *) Pre-classification according results from CzHI (2011).

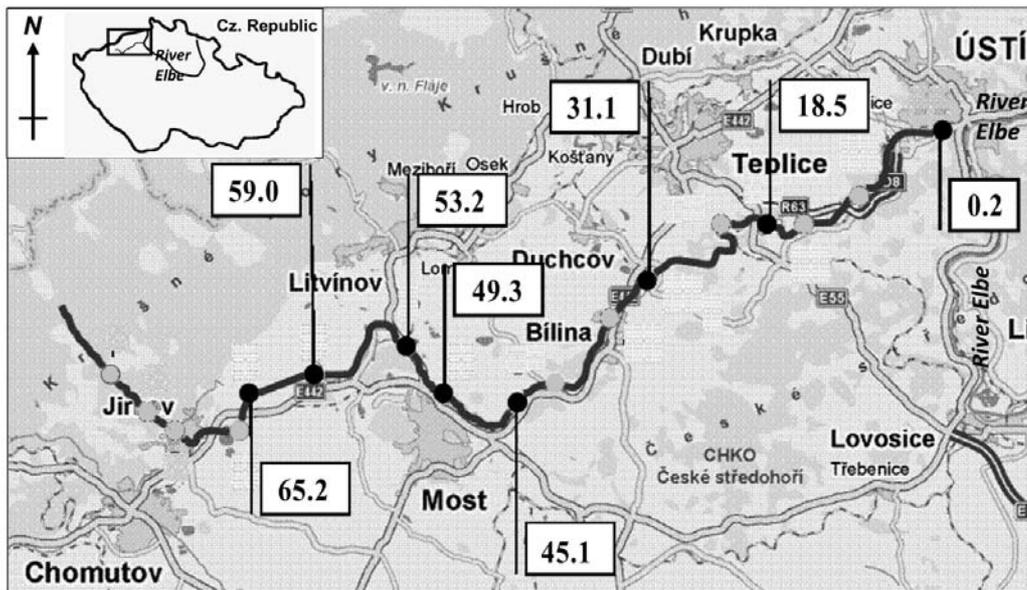


Fig. 1. Positions (according to river km) of the eight sample sites along the Bílina River (see also Table 1). Arrows indicate waste water treatment plants.

however, as low levels of pollution are still received from within the catchment area, as seen from monitoring data of the local water agencies (CzHI 2011). At km 54.0, upstream of the site at km 53.2, outlets discharge from the Litvínov town WWTP and the Chemopetrol Litvínov Co. refinery, while some kilometres downstream (before the site at km 49.3), there is a large WWTP serving the town of Most. Further downstream, industrial and wastewater inflow decreases along the whole stretch up to the town of Ústí nad Labem (km 0.2).

Material and methods

Field work

Sampling took place over four campaigns from 2006 to 2008 (Table 1). Macroinvertebrates were collected by kick sampling (500 µm mesh size) according to EN 27828:1994. All mesohabitats (e.g., large cobbles, boulders, submerged macrophytes, debris) from both lotic and lentic habitats covering >5% of the substrate surface within a 100 m stretch were merged in the sample in order to avoid the effects of local variability on ecological traits and biotic indices (Brabec et al. 2004; Sychra et al. 2010). At each site, one three-minute sample was taken. All material was sieved (500 µm mesh size) and transferred to one or two 1 L bottles for transport to the laboratory. A solution of 37% formaldehyde was added to obtain a final concentration of approximately 5% for preservation. The relative abundance of easily identifiable taxa was noted in the field. In the laboratory, macroinvertebrates were identified to the lowest possible taxonomical level using keys by Sperber (1950), Wachs (1967), Brinkhurst (1971), Wiederholm (1983, 1986), Moller Pillot (1984a, 1984b), Rivošecchi (1984), Pitsch (1993), Sauter (1995), Gittenberger et al. (1998), Glöer & Meier-Brook (2003), Vallenduuk (1999), Vallenduuk & Moller Pillot (1999), Klink (2002), Killeen et al. (2004), Neu & Tobias (2004), Bauernfeind & Humpesch (2001), Glöer (2002), Hölzel (2002), Janeček (2003), Langton & Visser (2003), Timm & Veldhuijzen van Zanten (2003), Eiseler (2005), Wilson & Ruse (2005), Lechthaler & Car (2005), Lechthaler &

Stockinger (2005), Orendt (2008) and a reference collection. The numbers of individual taxa were counted and classified in abundance classes according to AQEM protocols (AQEM 2004).

Physico-chemical data were measured at the same time as macroinvertebrate sampling (Table 2) using electronic instrumentation (WTW Ltd.). One-litre samples of water were taken on each sampling date for ion analysis, the water being kept cool during transport and then deep-frozen until chemical analysis. Inductively coupled plasma atomic emission spectroscopy (SpectroCiros CCD) following EN ISO 11885 (1998) was used for the analysis of Ca^{2+} , Mg^{2+} , Na^+ , Si_2^{4+} and total Fe, while ion chromatography (Dionex DX500) was applied for PO_4^{3-} , NO_3^- , NO_2^- , Cl^- and SO_4^{2-} .

Data analysis and statistics

Taxa abundance was estimated into seven classes (1 = single record to 7 = highly abundant) according to categories suggested by DIN 38410 (2004). Both metrics and ecological quality classes according to the WFD were calculated using the ecological running water assessment software tool in ASTERICS V.3.1 (www.fliessgewaesserbewertung.de; AQEM 2004; University of Duisburg-Essen 2008). Where statistical analysis and metric calculation required abundance data (e.g. distribution of feeding types, dweller types, stream type profiles using categories defined by Illies & Botosaneanu 1963), a transformation of the class values into numerical values was performed as recommended for the procedure in ASTERICS. Additional metrics, such as species richness, number of EPT (Ephemeroptera, Plecoptera, Trichoptera) taxa, BMWP (Biological Monitoring working party; Hellowell 1978) and the Czech Index of Saprobity (SI; CSN 75 7716, 1998), were calculated for description of community structure and organic pollution. A Principle Component Analysis (PCA; based on a variance-covariance matrix) was performed in order to reduce the large dataset and to highlight important physico-chemical variables (using scores from the first two factors as criteria). Data were log-transformed in order to avoid dominant influences of variables with high values. Correspondence analysis (CA) of the taxonomic community was undertaken in order to

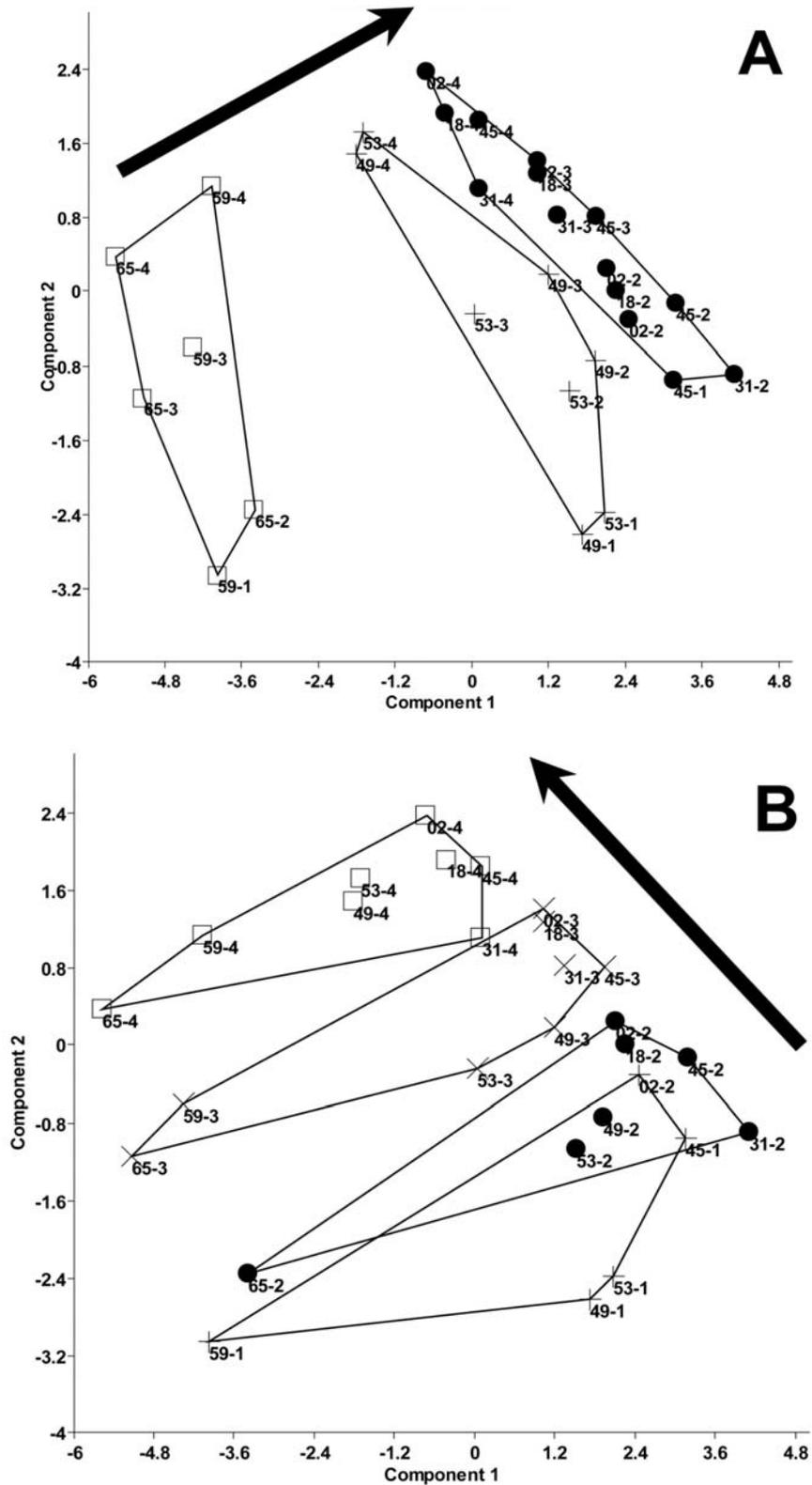


Fig. 2. Plot of sample sites (different dates) according to field measurements and ion concentrations using the first two components of a PCA (1st component: 57.6% of total variance; 2nd component 16.6%; 3rd component: 8.6% [not included in the plot]) showing geographical distribution. Explanation of codes: 59-1 = sampling site river km 59.0 on the first campaign (June 2006), 59-2 = sampling site river km 59.0 on the second campaign (September 2007). etc. A – Different symbols represent reference, impacted and non-categorised sites, respectively. + = polluted sites, □ reference sites, ● site km 45.1 and 31.1. B – Different symbols represent the different sampling dates of the four campaigns. Arrow: direction of first to last sampling campaign, illustrating seasonal variation. □ April 2008, × October 2007, ● September 2007, + June 2006.

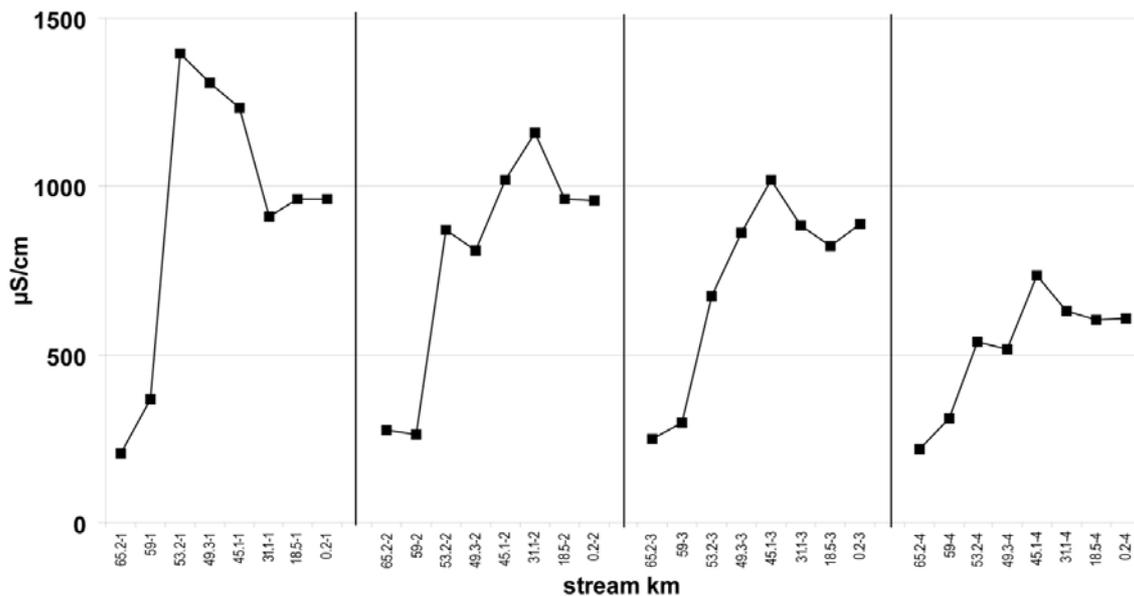


Fig. 3. Conductivity along the study reach over four separate campaigns (June 2006, Sept 2007, Oct 2007, Apr 2008). Explanation of codes: 59-1 = sampling site river km 59.0 on the first campaign (June 2006), 59-2 = sampling site river km 59.0 on the second campaign (September 2007), etc.

find relevant taxa and to reduce the taxa list, thereby allowing observation of further relationships with environmental variables. Canonical Correspondence Analysis (CCA), using untransformed data, was performed in order to study relationships between biological metrics and abiotic parameters from sampling sites during different sampling campaigns. Spurious correlations between environmental variables were traced by applying partial correlation analysis. The Kruskal-Wallis test was used to identify significant differences between metrics, variables and sites, while the Mann-Whitney test was applied for detailed pair-wise tests between single sample sites. The Wilcoxon Signed Rank test was used to search for differences between dates at the sample sites, and Spearman's rank correlation (1-tailed) was used to test for bivariate correlation. PCA, partial correlations calculated with SPSS, CCA, CA, and statistical tests were calculated using the PAST software package (version 2.07, 2011; Hammer et al. 2001)

Results

Physico-chemical characteristics and ions

An overview of the data measured is given in Table 2. PCA revealed both geographical and temporal differences in the data. The geographical gradient is represented by a clear separation in the position of the reference sites (km 65.0, 59.0) from impacted sites (km 53.2, 49.3) during all four sampling dates (Fig. 2A). Differences between the impacted sites and sites downstream (km 45.1 to km 0.2) are less striking, indicating similar environments. Temporal variation is illustrated by a second PCA perspective (Fig. 2B), which shows a steady unidirectional change from the first to the last sampling date for all sites (shown as an example in Fig. 3). The scores (Table 3) identify sulphate, conductivity, and a number of other ions as principle contributors to the first factor (55.4% of cumulative

Table 3. Field measurement and ion concentration loading for the PCA.

	Component			
	1	2	3	4
Variance (%)	57.6	16.6	8.6	7.0
SO ₄ ²⁻	0.946	0.241	0.132	-0.040
Conductivity	0.898	0.330	0.081	-0.151
Ca ²⁺	0.895	0.018	0.361	0.183
Mg ²⁺	0.870	0.026	0.300	0.249
Cl ⁻	0.830	0.231	0.260	-0.0892
Na ⁺	0.824	0.430	0.135	-0.210
NO ₂ ⁻	0.205	0.912	0.130	0.032
O ₂	-0.317	-0.730	-0.409	0.192
PO ₄ ³⁻	0.366	0.108	0.843	-0.160
NO ₃ ⁻	0.188	0.380	0.797	0.199
Si ⁴⁺	0.321	-0.052	-0.085	0.871
Total Fe	0.356	0.034	-0.094	-0.839

variance), suggesting that the inflow of inorganic loads at km 53.2 is the main contributor to pollution on the river. Nitrite and oxygen contributed most to the second factor; however, this explains far less of the variance (16.6%) than the first factor. The third contribution factor, which contained relatively high levels of phosphate and nitrate, explained a relatively small level of variance (8.3%). The first and strongest PCA factor can be summarised as a "salinity" factor, while the second and third factors are "organic pollution" factors, contributing less to variance in the sample dataset and having a lower gradient. The longitudinal distribution of the compounds and parameters measured shows a clear increase in concentrations of almost all chemical parameters from reference to impacted sites. Conductivity, an indicator of mineral load, was highest down-

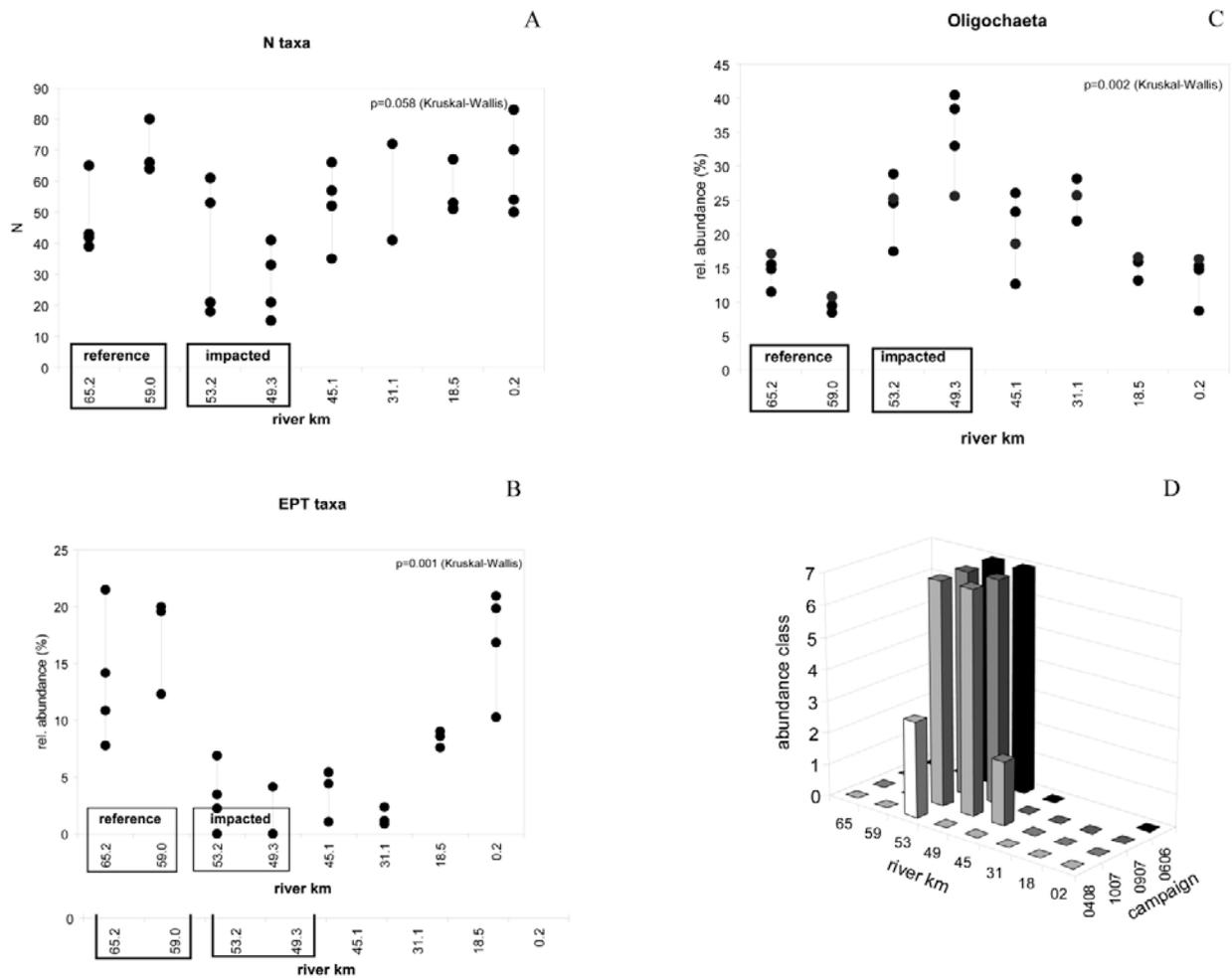


Fig. 4. A–C. Taxonomic metrics for the investigated sites along the Bílina River (in river km). Each dot represents the value of one campaign at a given site. D – Abundance of *Chironomus riparius* (see methods section for classes) on different sampling dates.

stream of the km 49.3 impacted site (Fig. 3), while calcium, magnesia and sulphate all showed a further increase at the km 53.2 and km 49.3 impacted sites. This pattern was observed during all campaigns, as revealed by the PCA plot (Fig. 2). Interestingly, elevated values of phosphate were also found at the reference sites during the first two campaigns. The data for phosphate, however, varied greatly over time and, in April 2008, values for phosphate at the reference sites were also the lowest observed. Oxygen concentrations were higher at the first impacted site (km 53.2) than at the reference sites upstream, but dropped towards the second impacted site at km 49.3 (Table 2). Measurements of temperature were excluded from the multivariate evaluation as the values were generally dependant on season and, therefore, not useful for further analysis. A separate analysis, however, showed small but significantly higher values at the impacted sites compared to the km 65.0 reference site on each sampling date. At km 18.5, significantly lower temperature values were observed than at km 45.1, and significantly higher values than at the km 65.0 reference site ($P < 0.05$; Wilcoxon test).

pH did not differ much around neutral values (7.47 ± 0.26 SD) between the sites suggesting that this sys-

tem is well buffered by the geochemical background or organic pollutants. Thus, mobilization of toxic heavy metals due to acidity does not play a major role in the pollution, here.

Metrics and response of selected taxa along the river stretch

A total of 309 taxa were recorded during the four sampling campaigns (see Appendix 1). An assessment of ecological status derived from the macroinvertebrate community indicated little variation between sampling sites: classes 3 (moderate) or 4 (poor) for all samples. The impacted sites (km 53.2 and 49.3) did not differ substantially from either the reference or other sites. Only between reference site km 59.0 and the final site (km 0.2) was a “moderate” status reached.

Taxa richness (Fig. 4A) dropped remarkably between reference site km 59.0 and the downstream impacted sites at km 53.2 and 49.3, narrowly missing statistical significance ($P < 0.051$; Mann-Whitney; $n = 29$). Compared to the other sites, taxa numbers at km 53.2 showed great variation (from 18 in September 2007 to 61 in April 2008). Taxa richness at the second impacted site (km 49.3) showed less variability and differed significantly only from the final station at km 0.2.

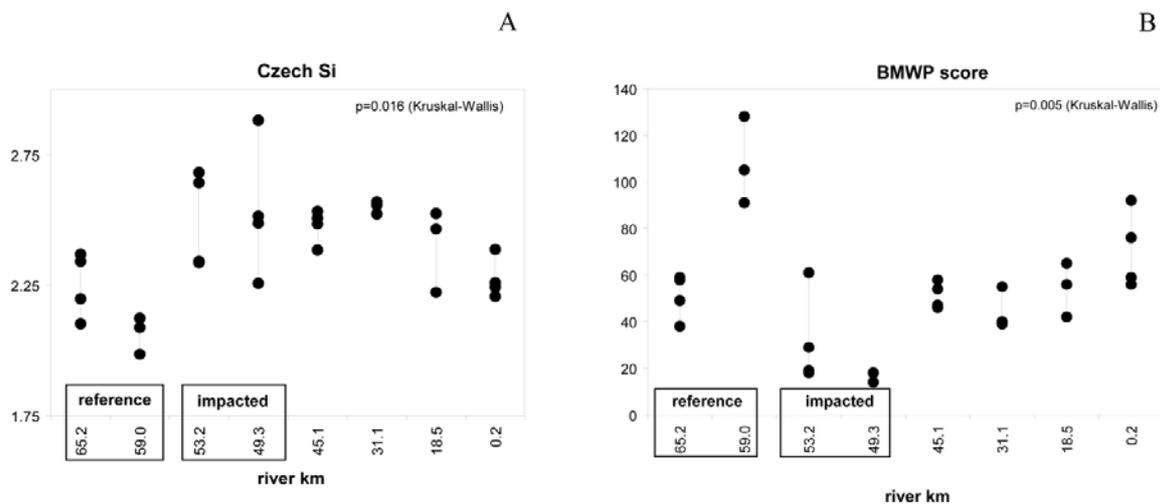


Fig. 5. Assessment and functional metrics for the investigated sites along the Bilina River (in river km). Each dot represents the value of one campaign at a given site.

The difference to reference km 59.0 was close to significance ($P < 0.051$, Mann-Whitney). At km 45.1, higher taxa richness values were regularly recorded (median = 55). Downstream, richness dropped (median = 41) but increased again to higher values towards the mouth of the river at km 0.2 (median = 62).

Total EPT abundance (Fig. 4B) clearly followed the same pattern ($P < 0.001$; Kruskal-Wallis), while oligochaete abundance generally showed the opposite pattern ($P < 0.003$; Kruskal-Wallis; Fig. 4C). Differences in oligochaete abundance between reference site km 65.2 and km 0.2, and between the impacted sites at km 53.2 and 49.3, were significant ($P < 0.030$; Mann-Whitney). The difference between reference site km 59.0 and the impacted sites was close to significance ($P < 0.051$; Mann-Whitney). A similar distribution was found for *Chironomus riparius*, which is an indicator for pollution (Fig. 4D). This species was very abundant at impacted sites km 53.2 and km 49.3, but showed only scattered occurrence at km 45.1 ($P < 0.014$; Kruskal-Wallis). On the last sampling date, however, only low abundance was recorded and then only at site km 53.2.

Water quality measurements varied significantly along the study stretch. SI (Fig. 5A) increased from reference site km 59.0 to impacted km 53.2 (and further downstream of that site), though a significant difference was only recorded between reference site km 65.0 and polluted site km 45.1 ($P < 0.030$; Mann-Whitney). BMWP values (Fig. 5B) showed clearer significant differences between sites than SI ($P < 0.004$; Kruskal-Wallis). The difference between both reference sites and the second polluted site at km 49.3 were also significant (both $P < 0.043$; Mann-Whitney), as was the drop from the first polluted site at km 53.2 to the second at km 49.3 ($P < 0.037$; Mann-Whitney).

Gatherers/collectors, grazers/scrapers and predators (feeding guilds), and phytal, lithal and pelal dwellers (habitat guilds) explained most of the variance in the first component of PCA analysis (1st component: 81.5%, 2nd component: 11.6%; Table 4). Aside from

Table 4. Functional group (guild) loadings of the first two PCA components from all dates and sites (initial data: dominances in %).

	Component	
	1	2
Explained variance (%)	81.5	11.6
Metric		
Gatherers/Collectors	227.20	20.64
Grazers/Scrapers	97.88	-25.04
Predators	73.90	-26.06
Passive filter feeders	32.00	-17.58
Active filter feeders	28.68	-1.07
Shredders	18.04	-1.11
Phytal dwellers	158.70	-46.39
Lithal dwellers	106.65	-40.28
Pelal Hdwellers	95.34	21.66
Psammal dwellers	73.34	12.81
Akal dwellers	45.06	-1.09

phytal dwellers and grazer/scrapers, all guilds showed significant changes in community dominance ($P < 0.05$; Kruskal-Wallis; $n=28$; pelal and lithal dwellers are shown as examples in Fig. 5).

When taxa were used for PCA instead of metrics (only taxa with a frequency < 3 and abundance < 5 , resulting in around 100 taxa), total variance of the 1st component was much lower and little difference was observed between components (1st component: 15.7%; 2nd component: 12.6%; 3rd component: 10.3%), indicating a weak gradient in the data. Nevertheless, the three groups (reference sites, polluted sites and others) were still clearly separated (plot not shown).

Summing up, in comparison with the reference sites at km 65.2 and km 59.0, both the taxonomic and functional community changed significantly following inflow of chemical industry wastewater at km 53.2. Eco-

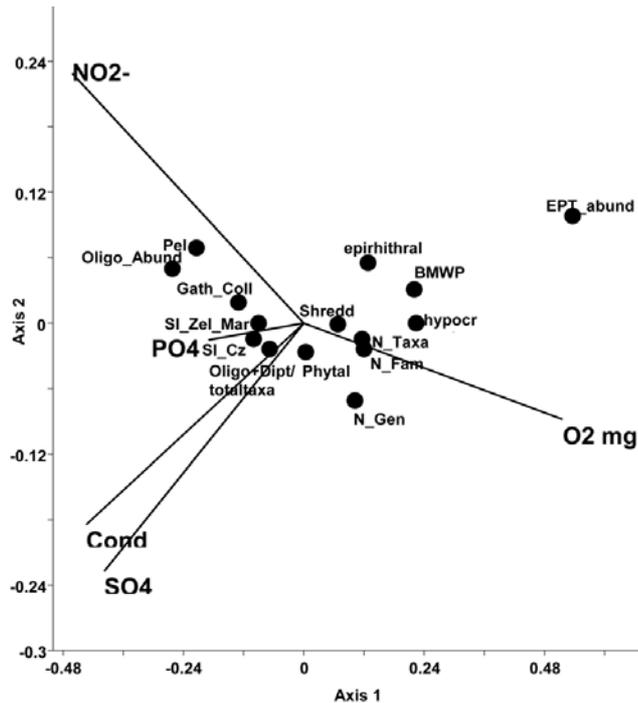


Fig. 6. Plot of CCA axis scores (axis 1: 90.56% of explained variance; axis 2: 4.97%; axis 3: 3.23%) using metrics. Abbreviations: BMWP – BMWP score; epirhithral – share (percentage) of epirhithral habitat type in the community; EPT_abund – total abundance of all Ephemeroptera, Plecoptera, Trichoptera taxa; Gath_Coll – share (percentage) of gatherer and collector feeding type in the community; hypocrc – share (percentage) of hypocranal habitat type in the community; N_Fam – number of families; N_Gen – number of genera; N_Taxa – number of (high resolved) taxa; Oligo_Abund – total abundance of Oligochaeta; Oligo+Dipt/totaltaxa – share of abundances of (Oligochaeta + Diptera)/abundance of all taxa; Pel – share (percentage) of pelal habitat type in the community; Shredd – share (percentage) of shredder feeding type in the community; SI_Cz – Czech index of saprobity; SI_Zel_Mar – index of saprobity according Zelinka & Marvan.

logical functions changed slightly downstream of the heavily impacted sites, indicating a level of recovery, but conditions declined again after km 41.8, only increasing once again at the end of the river (km 0.2). At this latter site, the distribution of ecological functions (i.e., feeding or dwelling guild, taxonomic diversity and SI) were similar to those at reference sites km 65.2 and km 59.0, upstream of the pollution source at Litvínov (km 53.2).

Relationships between metrics and environmental variables

Though many significant correlations were observed between metrics and abiotic variables, among metrics, and with SI when bivariate tests were used, a partial correlation procedure indicated that most were spurious, only that between pelal dweller dominance and NO_2^- proving to be truly significant. Due to autocorrelations and crosswise factor influences in the dataset, multivariate treatments proved most useful for studying the relationships between environmental variables and metrics or taxa. For this reason, a CCA (Fig. 6) was used to

show the relationships between biological metrics and physico-chemical parameters and ion concentrations. The CCA dataset consisted of (1) conductivity, SO_4^{2-} , O_2 concentrations, NO_2^- and PO_4^{3-} as environmental variables (indicated as important by PCA analysis; see Table 3), (2) those metrics significantly correlated to abiotic parameters ($P < 0.05$; Spearman rank correlation), and a reduced taxa list containing only taxa with a frequency >2 in all samples and a score >0.7 in a preceding CCA including all taxa.

The CCA (Fig. 6) revealed a strong affinity between EPT abundance, BMWP score, shares of hypocranal dwellers, and number of taxa of different levels to O_2 concentrations. Pelal dweller, gatherer/collector dominance and oligochaete abundance were plotted close to the NO_2^- variable. The shares of Oligochaeta and Diptera in relation to total taxa abundance had an affinity to the “salinity” determinant (described by SO_4^{2-} and conductivity). The SI score was close to PO_4^{3-} concentrations. The first axis of the CCA scores explained 90.57% of total variance, which is very high.

A plot of sample sites did not reveal any grouping pattern, whether by season or position along the stretch (figure not shown).

When taxa were used for the CCA instead of metrics, the first axis explained only 40.51% of total variance, the second 32.03%, and the third 20.03%. The triplot (Fig. 7), however, indicates a clear grouping of reference, impacted and non-categorised sites. The reference sites were connected to the “organic load determinant” in the plot, being associated with the O_2 determinant and opposite to the NO_2^- . Some samples from km 18.5 and site 0.2 were also close to this group. A further group comprising the two sites downstream of the impacted sites (km 45.1 and km 31.1) was linked to the “salinity determinant”, represented by high values of conductivity and SO_4^{2-} . The clearly separated group of impacted sites was situated close to the “organic load determinant”, also indicated by its opposition to the PO_4^{3-} variable.

Taxa distribution according to site (Fig. 7) indicates that *C. riparius* was strongly linked to poor environmental conditions at the polluted sites, while *Hydropsyche angustipennis* and *Glyptotendipes pallens* (both filter feeders) showed a preference for the reference sites, not being recorded at other sites. Scores for the first axis of the CA, while significantly correlated to NO_2^- ($r_S = 0.402$; $P < 0.04$) showed a somewhat stronger correlation to conductivity ($r_S = 0.674$; $P < 0.001$), suggesting that the taxonomic community reflected the ion concentration (“salinity determinant”) gradient better than the “organic determinant” (though its contribution should not be neglected).

Discussion

Abiotic features

Analysis of physico-chemical parameters and ion concentrations revealed both temporal and local gradients.

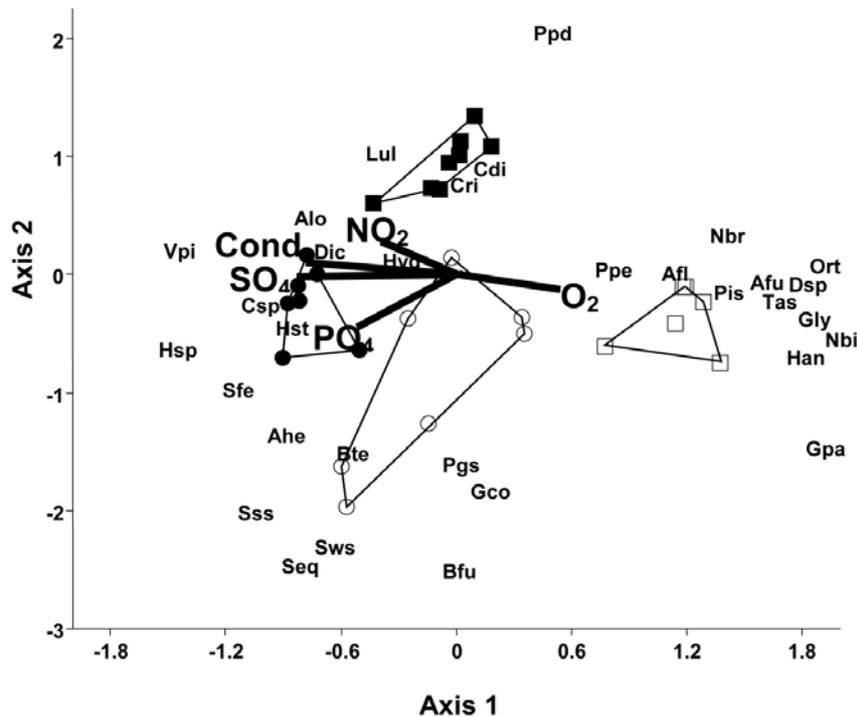


Fig. 7. Plot of CCA axis scores (axis 1: 40.5% of explained variance; axis 2: 32.1%; axis 3: 20.0%) using taxa (taxlist reduced). ■ polluted sites, □ reference sites, ● site km 45.1 and 31.1, ○ site km 18.5 and 0.2. Afl – *Ancyclus fluviatilis*, Afu – *Anabolia furcata*, Ahe – *Alboglossiphonia heteroclita*, Alo – *Ablabesmyia longistyla*, Bfu – *Baetis fuscatus*, Bte – *Bithymia tentaculata*, Cdi – *Chaetogaster diaphanus*, Cri – *Chironomus riparius*, Csp – *Chironomus* sp., Dic – *Dicrotendipes* sp., Dsp – *Diamesa* sp., Gco – *Glossiphonia complanata*, Gly – *Glyptotendipes* sp., Gpa – *Glyptotendipes pallens*, Han – *Hydropsyche angustipennis*, Hsp – *Haliphus* sp., Hst – *Helobdella stagnalis*, Hyd – *Hydra* sp., Lul – Lumbiculidae gen. sp., Nbi – *Neureclipsis bimaculata*, Nbr – *Nais bretscheri*, Ort – Orthoclaadiinae gen. sp., Pgs – Psychodidae gen. sp., Ppe – *Paratanytarsus penicillatus*, Seq – *Simulium equinum*, Sfe – *Spirosperma ferox*, Sss – *Simulium (Simulium)* sp., Sws – *Simulium (Wilhelmia)* sp., Tas – Tanyptodinae gen. sp., Vpi – *Valvata piscinalis*.

Both conductivity and other ions followed this pattern, at least concerning changes in water quality at the impacted sites. Levels for O_2 and ions of N and P compounds did not increase downstream, however, but decreased towards the end of the study stretch. As with conductivity, both compound load and differences tended to decline between the first and last sampling campaign, suggesting a general reduction in pollution after the inlet and from the first to last sampling date. Our results suggest two main pollution sources, one deriving from “salinity” loading and the other from organic loading. The organic pollution comprised NO_2^- , NO_3^- and PO_4^{3-} variables, in addition to O_2 , with NO_2^- ions and O_2 showing higher statistical importance than NO_3^- and PO_4^{3-} (PCA) and a stronger significant correlation for O_2 with NO_2^- than NO_3^- . As NO_3^- may have originated from mineral wastewater and NO_2^- is a decomposition product of NH_4^+ derived from organic wastewater, we used NO_2^- as the descriptor for organic pollution during further evaluation.

Our data also indicated that the reference sites were affected by a certain amount of organic pollution. Due to the relatively low number of sampling dates, however, we were unable to smooth extreme values and outliers and, therefore, all physico-chemical data should be interpreted with care. The data were, however, sufficient to allow elaboration of plausible interpretations of the relationships between physico-chemical and biological data.

Biotic metrics and biota

Our data were in harmony with previous findings in literature, i.e. that a number of frequently used metrics, even one as simple as taxa richness, show significant responses to pollution and recovery. Resh et al. (2000) has stated that richness metrics were most accurate in detecting impairment, while Lenat & Penrose (1996) have shown that EPT taxa richness is even more stable and predictable than total taxa richness. Compared to these, BMWP, being based on macroinvertebrate family level, is likely to be less sensitive to ecosystem change than taxa richness metrics at the species level, although the former showed a clear response, in our study.

Of the functional metrics used, only correlation of NO_2^- and pelal dwellers proved to be positively significant. High pelal dweller dominance occurred at polluted sites, and on some dates, but was also comparable to that at the reference sites during the last sampling campaign, when these sites were least polluted. This suggests that habitat distribution of pelal dwellers may also be connected to the level of pollution rather than to locally consistent morphological structures alone, i.e., high organic load can result in increased organic sedimentation. Moreover, the distribution of lithal dweller dominance was also significant; suggesting that there may have been more morphological differences than the protocols used in this investigation were capable of detecting.

While all metrics used reflected levels of organic

pollution adequately, no dramatic differences in SI were noted over the stretch investigated, though levels were statistically significant. For example, SI confirmed findings from chemical measurement and indicated that the reference sites were also affected by organic pollution. The stronger correlation of SI with NO_2^- rather than other abiotic variables ($r_S = 0.703$; $P < 0.001$) suggests that the functional community was responding directly to an “organic pollution determinant” rather than a “salinity” determinant. The taxonomic community, on the other hand, showed a somewhat stronger relationship to the “salinity determinant”, as indicated by a stronger correlation to conductivity than to NO_2^- . This suggests that the taxonomic community may better reflect the “salinity determinant” than the functional community. Taxonomic composition and abundance determine the functional community in the analysis, however, whereas its functioning may be more complex. Dominance of *C. riparius*, for example, at polluted locations, e.g. km 53.2, indicates not only low competition caused by a lack of other species (indirect indication) but has also been found to be an effect of growth stimulation caused by the combined appearance of both organic and toxic pollution (direct indication), as found by Stuijzand et al. (1996) in studies from the Meuse River.

The fact that the taxonomic community and functional metrics respond differently to different types of environmental pressure has also been addressed by other authors, though with differing results. Pinel-Alloul et al. (1996) found that taxonomic and functional community indices followed a pollution gradient indicated by chemical compounds in different ways. Moreover, both indices stressed different aspects of macroinvertebrate community structure, with a clear response to morphology. Further, during investigation of macroinvertebrate communities from differing morphological areas (lentic and lotic), Brabec et al. (2004) has shown that assessment results differ under the same level of pollution. While Simiao-Ferreira et al. (2009) found a response to pollution that was clearly caused only by sewage; it required low variation in morphological features. In our study, we also found no substantial morphological differences between the sites investigated, strongly suggesting that pollution was the main influence on community structure. This finding is similar to that of Adamek & Jurajda (2001).

We found no similar reference in the literature to the degree in which the “salinity factor” and “organic factor” were indicated by the taxonomic and functional communities, respectively, in this study. However, Piscart et al. (2005) did observe a decrease in macroinvertebrate taxonomic richness in a small stream affected by a salinity gradient and, in terms of ecological function, there followed a slight change in the relative abundance of invertebrate feeding groups. Blasius & Merritt (2002), when investigating the effects of road salt on stream communities, found no substantial impact on either taxonomic or functional community. In their study, however, conductivity levels were >2 fold higher than

in our study, suggesting that only highly tolerant taxa were to be found in the waters studied. Other authors have, however, reported clear effects (e.g., Crowther & Hynes 1977; Dickman & Gnochauer 1978; Demers & Sage 1990). In an early study, Turoboyski (1959; cited in Necchi Júnior et al. 1994) studied pollution caused by salinity and organic matter, though using microbial communities. More recent studies have monitored the complex pollution patterns caused by industrial activities, both with and without municipal sewage run-off (Mooraki et al. 2009; Arimoro et al. 2011). In these studies, however, differentiation between “salinity” load and organic wastewater was either not detected or not analysed for. Tho et al. (2006) were able to elaborate separate influences from shrimp monoculture (pollution resulting in eu- to hyperhaline salinity) and human influence (organic pollution), but did not discuss indication by macroinvertebrates. Whereas Braukmann & Böhme (2011) were able to show clear changes in the taxonomic macroinvertebrate community due to industrial salt contamination (rather than to other types of chemical variable or organic pollution) in the Werra River (Germany), variation and level were not marked. Once again, however, comparison with our results is limited as ecological function was not analysed for and maximum chloride concentrations were around 20-times higher and maximum conductivity 6-times higher than in our study.

In contrast to the above, our results showed that the “organic pollution determinant” had a stronger effect on the functional community than the taxonomic (though this does not mean that there was no taxonomic community response). Further, CCA also indicated that certain taxa were clearly related with the organic pollution parameters O_2 and NO_2^- . With less organic pollution, however, the “salinity pollution determinant” was better reflected by the taxonomic community. These results may be a result of the particular character of the study stretch and reflect the unique pattern of pollution.

References vs. impacted sites

As indicated by the SI and BMWP values above, the first reference site appears to have been somewhat degraded by chemical pollution. This may have been caused by the outflow of higher amounts of organic material and other chemical and metabolic compounds at site km 65.2 than at the next site downstream. The higher SI value at km 65.2 this caused must make the use of this site as a true “reference” questionable. Regarding dweller type dominance, total taxa number, EPT taxa number, SI and BMWP, the site was ecologically similar to those near the river’s mouth at sites km 18.5 and km 0.2. In consequence, the site at km 59.0 is the only true reference site for this study.

A range of metrics showed highest values at the km 53.2 and km 49.3 impacted sites. At the latter site, the results at the end of the study period (April 2008) were less different from the other sites than in the previous sampling dates, suggesting reduced pollution dur-

ing this last sampling period. While this corresponds with the measurements of conductivity and other parameters recorded in the field (Fig. 2B), more data are required to provide clearer evidence of a temporal trend due to a sustainable reduction in wastewater influx or exceptional dilution by rainfall. Our data are sufficient, however, to outline reactions of the macroinvertebrate community to changes in environmental conditions. As such, the results presented here can be used as a reference for assessing changes in environmental impact from multiple pollution sources, being more detailed and in a finer form than those through assessment according to the EU-WFD.

Conclusions

The results of our analysis show that pollution by organic wastewater had more impact on the macroinvertebrate community than an increase in ion concentration caused by mineral wastewaters, though the taxonomic community appeared to respond more sensitively to variations in “salinity” concentration than did ecological function. As functional metrics and the taxonomic community responded in different ways to various pressures, the analysis of both is necessary to diagnose and prioritise multiple stressors. As indicated by data from the local water agencies (CzHI, 2011), there is a high likelihood of high levels of both industrial and municipal pollution from compounds not analysed in this investigation, and from toxic organochemical compounds derived from pharmaceutical and industrial activities in particular. Extensive investigations have been carried out in the past, however, and data gathered that can be used in further studies that will allow us to elucidate community response to such organochemical compounds.

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Appendix 1. List of taxa recorded and their abundance in classes according to DIN 38410 (2004): Abundance class 1 = 1–2 specimens per sample (single to scattered); abundance class 2 = 3–10 (sparse to several); abundance class 3 = 11–30 (medium density); abundance class 4 = 30–100 (fairly dense); abundance class 5 = 101–300 (abundant, dense); abundance class 6 = 301–1000 (very abundant, very dense); abundance class 7 = >1001 (heaps). Reference sites in **bold**, polluted sites in *italics*. Abbreviations: grp. – group; Ad. – adult; Lv. – larva.

	Date	2006-06-20	2006-06-20	2006-06-20	2006-06-21	2006-06-21	2006-06-21	2007-09-24	2007-09-24	2007-09-25	2007-09-25	2007-09-24	2007-09-24	2007-09-24	2007-10-24	2007-10-22	2007-10-22	2007-10-24	2007-10-23	2007-10-23	2007-10-23	2007-10-23	2008-04-23	2008-04-23	2008-04-23	2008-04-22	2008-04-22	2008-04-22	2008-04-22		
Site (stream km)		65.2	59.0	53.2	49.3	45.1	0.2	65.2	53.2	49.3	45.1	31.1	18.5	0.2	65.2	59.0	53.2	49.3	45.1	31.1	18.1	0.2	65.2	59.0	53.2	49.3	45.1	31.1	18.5	0.2	
Hydrozoa																															
<i>Hydra</i> sp.		7	3	4	3	4	2	4	.	.	5	5	1	1	2	.	.	.	1	.	4	3	3	3	1	2	
Turbellaria																															
<i>Dendrocoelum lacteum</i> (O.F. Mueller, 1774)		1	1	2	
<i>Dugesia lugubris</i> (Schmidt, 1861)		2	
<i>Dugesia lugubris/polychroa</i>		4	.	.	2	1	4	2	3	3	2	.	.	
<i>Dugesia</i> sp.		2	3	3	
<i>Dugesia tigrina</i> (Girard, 1850)		1	
<i>Polycelis tenuis</i> Ijima, 1884		1	
<i>Polycelis</i> sp.		1	
Turbellaria Gen. sp.		2	
Nematoda																															
Nematoda Gen. sp.		.	1	2	3	.	1	2	.	1	.	2	.	2	1	1	.	.	2	2	2	.	2	.	2	.	1	3	2	1	
Bivalvia																															
<i>Anodonta</i> sp.		1	.	
<i>Musculium lacustre</i> (O.F. Mueller, 1774)		.	.	.	1	1	.	.	
<i>Pisidium casertanum casertanum</i> (Poli, 1791)		2	.	
<i>Pisidium casertanum</i> sp. (Poli, 1791)		2	2	
<i>Pisidium henslowanum</i> (Sheppard, 1823)		2	3	
<i>Pisidium</i> sp.		2	2	.	1	1	3	.	.	.	1	2	.	5	2	.	.	.	2	3	1	1	4	.	.	.	2	1	.		
<i>Pisidium subtruncatum</i> Malm, 1855		1	2	.	2	3	2	
<i>Sphaerium corneum</i> (L., 1758)		3	2	3	2	.		
<i>Sphaerium</i> sp.		1	2	.	.	
Gastropoda																															
<i>Acroloxus lacustris</i> (L., 1758)		1	.		
<i>Ancylus fluviatilis</i> O.F. Mueller, 1774		1	.	.	1	1	2	.	4	1	2	1	.	
<i>Bithynia tentaculata</i> (L., 1758)		2	.	2	2	.	.	1	4	1	2	1	1	.	1	.	
<i>Gyraulus albus</i> (O.F. Mueller, 1774)		.	1	.	.	2	.	1	2	.	2	.	.	.	1	2	
<i>Physella acuta</i> Draparnaud, 1805		.	.	5	2	.	1	4	3	3	5		
<i>Physella</i> sp.		2	5	2	2		
Physidae Gen. sp.		.	3	
Planorbidae Gen. sp.		.	.	.	1	
<i>Potamopyrgus antipodarum</i> (J.E. Gray, 1843)		1	.	1	1	.	2	
<i>Radix auricularia</i> (L., 1758)		.	1	
<i>Radix balthica</i> (L., 1758)		4	2	
<i>Radix labiata</i> (Rossmassler, 1835)		1	
<i>Radix</i> sp.		2	
<i>Valvata piscinalis</i> ssp. (O.F. Mueller, 1774)		.	2	.	2	.	.	.	4	3	1	
Hirudinea																															
<i>Alboglossiphonia heteroclita</i> (L., 1758)		5	4	.	1	.	
<i>Erpobdella nigricollis</i> (Brandes, 1899)		1	3	2	3	3	.	
<i>Erpobdella octoculata</i> (L., 1758)		2	.	5	4	5	.	2	3	4	5	6	2	.	2	4	2	5	3	3	.	.	.	1	6	4	4	.	.		
<i>Erpobdella</i> sp.		2	2	2	.	
<i>Erpobdella vilnensis</i> (Liskiewicz, 1925)		1	.	.	
Erpobdellidae Gen. sp.		1	.	.	2	4	2	.	2	2	2	4	2	.	.	.	2	.	2	.	2	
<i>Glossiphonia complanata</i> (L., 1758)		2	1	.	.	2	1	1	1	1	4	.	.	.		
<i>Glossiphonia concolor</i> (Apathy, 1888)		
<i>Glossiphonia</i> sp.		2	2	.	1	.	
Glossiphoniidae Gen. sp.		2	
<i>Helobdella stagnalis</i> (L., 1758)		1	2	.	5	2	5	2	4	7	5	3	2	4	.	.	7	4	4	2	2	.	1	2	6	5	2	1	.		
<i>Hemiclepsis marginata</i> (O.F. Mueller, 1774)		.	.	.	1	
<i>Theromyzon tessulatum</i> (O. F. Mueller, 1774)		1	1	
Oligochaeta																															
<i>Aulodrilus plurisetus</i> (Piguet, 1906)		1	
<i>Chaetogaster diaphanus</i> (Gruithuisen, 1828)		2	4	3	3	3	.	3	3	3	1	
<i>Eiseniella tetraedra</i> (Savigny, 1826)		
Enchytraeidae Gen. sp.		.	2	.	.	3	1	2	.	2	
<i>Limnodrilus claparedeanus</i> Ratzel, 1868		.	3	2	3	.	3	4	4	2	3	2	3	3	4	.	.	.	3	3	2	2	3	.	.	.	

Appendix 1. (continued)

	Date	2006-06-20	2006-06-20	2006-06-20	2006-06-21	2006-06-21	2006-06-21	2007-09-24	2007-09-24	2007-09-25	2007-09-25	2007-09-24	2007-09-24	2007-09-24	2007-10-24	2007-10-22	2007-10-22	2007-10-24	2007-10-23	2007-10-23	2007-10-23	2008-04-23	2008-04-23	2008-04-22	2008-04-22	2008-04-22							
Site (stream km)		65.2	59.0	53.2	49.3	45.1	0.2	65.2	53.2	49.3	45.1	31.1	18.5	0.2	65.2	59.0	53.2	49.3	45.1	31.1	18.1	0.2	65.2	59.0	53.2	49.3	45.1	31.1	18.5	0.2			
<i>Hydropsyche pellucidula</i> (Curtis, 1834)		2			
<i>Hydropsyche pellucidula</i> grp.		2			
<i>Hydropsyche</i> sp.		2	6	5	5	7	2	5	4	2	2	3		
<i>Hydroptila</i> sp.		1	.	.	2	1	1	.	1	1	2	2	5		
Hydroptilidae Gen. sp.		1		
Leptoceridae Gen. sp.		2	2		
Limnephilidae Gen. sp.		4		
<i>Limnephilus</i> sp.		1		
<i>Molanna angustata</i> Curtis, 1834		1		
<i>Mystacides nigra</i> (L., 1758)		2		
<i>Neureclipsis bimaculata</i> (L., 1758)		4	4	2	3	1		
<i>Psychomyia pusilla</i> (F., 1781)		2	.	2	2	4		
<i>Tinodes</i> sp.		.	.	1		
Lepidoptera																																	
Lepidoptera Gen. sp.		1	
Megaloptera																																	
<i>Sialis lutaria</i> (L., 1758)		1	
Heteroptera																																	
Corixinae Gen. sp.		1	
Corixini Gen. sp.		1	
<i>Micronecta</i> sp.		2	2	.	1		
<i>Gerris lacustris</i> (L., 1758)		1	
Coleoptera																																	
<i>Elmis maugetii</i> Latreille, 1798		1	
<i>Elmis</i> sp.		1	1	
<i>Elmis</i> sp. Ad.		1	
<i>Haliplus fluviatilis</i> Aube, 1836		.	.	.	1	
<i>Haliplus immaculatus</i> Ad. Gerhardt, 1877		1	
<i>Haliplus</i> sp.		.	.	.	1	1	4	2	2	1	1	1	1		
<i>Hydraena</i> sp. Ad.		1	
<i>Limnius volckmari</i> (Panzer, 1793)		1	
<i>Orectochilus villosus</i> (O.F. Mueller, 1776)		3	2	
<i>Platambus maculatus</i> Ad. (L., 1758)		1	
<i>Platambus maculatus</i> Lv. (L., 1758)		2	
Diptera – Simuliidae																																	
<i>Prosimulium</i> sp.		1	
<i>Simulium (Nevermannia)</i> sp.		.	1	1	
<i>Simulium (Simulium)</i> sp.		2	
<i>Simulium (Simulium)</i> sp.		2	.	4	5	5	.	1	4	1	1		
<i>Simulium (Wilhelmsia)</i> sp.		.	.	.	2	2	1	1	5	4	2	2	
<i>Simulium angustipes</i> Edwards, 1915		.	.	.	1	
<i>Simulium equinum</i> (L., 1758)		5	4	2	
<i>Simulium erythrocephalum</i> (DeGeer, 1776)		.	2	2	2	6	2	2	5	6	3	4	3	.	.	1	4	3	1	4	3	.	.	2	2	3	2		
<i>Simulium lineatum</i> (Meigen, 1804)		2	2	
<i>Simulium morsitans</i> Edwards, 1915		2	
<i>Simulium ornatum</i> Meigen, 1818		.	4	.	.	2	.	.	5	2	1	.	2	1	.	2	1	.	1	2	.	.		
<i>Simulium ornatum</i> Meigen, 1818		1	
<i>Simulium reptans</i> (L., 1758)		.	2	.	4	
<i>Simulium</i> sp.		.	4	.	4	4	2	3	.	2	6	2		
Diptera – Chironomidae																																	
<i>Ablabesmyia longistyla</i> Fittkau, 1962		.	.	2	2	2	
<i>Brillia bifida</i> Kieffer, 1909		2	2	
<i>Brillia flavifrons</i> (Johannsen, 1905)		2	2	2	.	2	.	2	1	.	.		
<i>Brillia</i> sp.		2	
<i>Cardiocladius capucinus</i> (Zetterstedt, 1850)		2	2	.	2	
<i>Cardiocladius fuscus</i> Kieffer, 1924		3	.	.	2	
<i>Chaetocladius piger</i> (Goetghebuer, 1913)		3	.	2	.	.	.	
Chironomini Gen. sp.		2	
<i>Chironomus nuditarsis</i> Keyl, 1961		1	
<i>Chironomus nudiventris</i> Ryser, Scholl et Wuelker, 1983		3	1
<i>Chironomus plumosus</i> (L., 1758)		.																															

