

Macroinvertebrate herbivores and epilithon algae community of a stream affected by mineralized heated mining water inflow

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Abstract – The main aims of this study were to analyse the effect of a mineralized heated water inflow from a uranium ore processing plant on the abundance and diversity of epilithon and macroinvertebrate herbivores as well as to identify changes in functional feeding groups. In total, four sampling sites were selected. One site was on the mining water tributary and three sites in a recipient natural stream (the Nedvědička River, Czech Republic): one of them upstream and two downstream from the mining effluent. Samples were taken monthly during the period 2008–2009. The number of species in the epilithon was higher downstream from the tributary and we also recorded a small increase in density. We observed significant changes in the taxonomical composition of epilithon and macroinvertebrate communities downstream from the tributary. Taxa richness of macroinvertebrates was similar between sampling sites. A small drop in macroinvertebrate abundance immediately downstream from the tributary was followed by a significantly elevated number of herbivores, especially scraper-collectors, at the more downstream site. Alteration of the species composition and abundance of herbivorous macroinvertebrates in the Nedvědička River could be explained by a combination of a change in food availability and the physico-chemical properties of the water.

Key words: Epilithon / functional feeding groups / herbivores / macroinvertebrate communities / thermal pollution

Introduction

Freshwater ecosystems are affected by various anthropogenic impacts. One of these is discharge from mining and industrial cooling waters. First, these effluents alter the temperature regime of streams, which is a key determinant of biotic community composition and function (Hogg and Williams, 1996; Živić *et al.*, 2006; Haidekker and Hering, 2008).

The other effect of mining water can be a change in the chemical quality of a recipient water body, such as increased salinity, acidity and heavy metal concentration (*e.g.*, Hamsher *et al.*, 2004; Merovich and Petty, 2007; Pond *et al.*, 2008; Timpano *et al.*, 2015), and/or a change in discharge and habitat quality (Nedea *et al.*, 2003).

A unique situation exists on the Nedvědička River in the Czech Republic, where decontaminated mining water and treated warm wastewater from a chemical ore processing plant flow into a small tributary, allowing us to study small-scale thermal pollution (1.8 °C on average)

on a small spatial scale. Nevertheless, the mining water tributary differs from the Nedvědička River, by having not only a higher temperature but also higher conductivity, pH and amounts of inorganic carbon on the one hand, and lower nutrient and oxygen levels on the other. This situation makes the influence on the aquatic biota more complex and some caution in the interpretation of results is needed. Fortunately, the mining water tributary did not significantly affect the Nedvědička River flow rate and substratum composition, so we can observe the direct effect of the mining water tributary without changes in stream morphology.

Spatiotemporal variations of water temperature directly and strongly influence the biological response of epilithon (*e.g.*, Descy and Mouvet, 1984) and the direct or indirect biological response of macroinvertebrates (*e.g.*, Lakly and Mc Arthur, 2000; Živić *et al.*, 2006).

Studies in areas receiving thermal effluent have found that the biomass of epilithon increased in heated areas (Hickman, 1974; Squires *et al.*, 1979; Eloranta, 1982; Descy and Mouvet, 1984). Patrick (1971) found that temperature increases smaller than 14 °C increased the

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biomass of temperate epilithon communities, particularly at low ambient temperature. However, temperature increases near the upper end of the range of tolerance (ca. 32 °C) caused the algal biomass to decrease. In addition, Brock (1970) postulated that increases in temperature should increase epilithon biomass accumulation in habitats at temperatures < 25 °C, but not in those > 25 °C, where thermophilic taxa should already be close to their optimum for growth. Species richness and diversity have been found to increase with temperatures up to 25–30 °C, then decrease above 30 °C, as species-rich diatom flora are replaced by fewer species of green algae or cyanobacteria (Patrick, 1969; Vinson and Rushforth, 1989).

Thermal discharges can also indirectly change algal biomass and composition by affecting the growth, emergence, biomass and composition of grazers (Lamberti and Resh, 1983, 1985). The presence of herbivores almost always has a negative effect on algal biomass, but the exact effect depends on the density and morphology of the herbivore (Steinman, 1996). However, algal biomass can increase even in the presence of herbivores if there is a nutrient supplement, which outweighs the negative effect of grazing (Samways *et al.*, 2015).

The direct effect of temperature alteration on macroinvertebrates is likely to differ between species, depending on their existing environment and life histories, and their ability to adapt. Many studies have confirmed that the main negative effect of thermal pollution on macroinvertebrates is to decrease the abundance and/or diversity of EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) (Adámek and Obrlík, 1977; Poff and Matthews, 1986; Voelz *et al.*, 1994; Lakly and McArthur, 2000; Nedeau *et al.*, 2003) or insect taxa completely (Živić *et al.*, 2013). Chironomidae are considered the most resistant insect group to thermal pollution, according to previous studies (Živić *et al.*, 2013). On the other hand, Hogg and Williams (1996) recorded a decrease in Chironomidae density, especially Orthocladiinae, even under low thermal stress (2–3.5 °C). Thermal polluted streams are often characterized by the dominance of non-insect macroinvertebrate taxa like Clitellata, Mollusca or Ostracoda (Taylor and Dykstra, 2005).

High sulphate-related conductivity, which is often associated with mining water, is known to have a negative effect on macroinvertebrate diversity (Batty *et al.*, 2005; Timpano *et al.*, 2015) and only a few groups (Clitellata, Diptera) can occur in extremely high conductivity conditions (Dumnicka and Galas, 2006). High or low pH usually has a negative effect on macroinvertebrate diversity (Coimbra *et al.*, 1996; Merovich and Petty, 2007). pH in the range 5–9 has a minimal direct effect on macroinvertebrates, but it increases the toxicity of metals (Hawkes, 1979) and influences processes such as decomposition (Petrin *et al.*, 2008) in aquatic environments.

The aims of this study were to determine how the mineralized heated mining water tributary affects abundance, taxa richness and composition of communities

of epilithic algae and bacteria and herbivorous macroinvertebrates. We hypothesized that (i) the abundance of epilithon would increase with temperature and community composition would be different between the affected and unaffected sampling sites, (ii) the abundance of some more sensitive herbivorous taxa would decrease, (iii) there would be a shift in community composition between the affected and unaffected sampling sites and (iv) more tolerant macroinvertebrate groups, especially scraper-collectors, would increase downstream from the mining water tributary.

Materials and methods

Study site

The Nedvědička River is a second-order stream located in the Bohemia-Moravian Highland in the Czech Republic (49°28'53"N, 16°14'00"E). It runs through the village of Rožná, where uranium has been mined since 1958. Decontaminated mining water is used as a coolant in a chemical uranium ore processing plant and subsequently discharged continuously into the Nedvědička River through a small tributary (0.06 m³.s⁻¹). For our study, four sampling sites were chosen: three on the Nedvědička River (US, DS1 and DS2), and one on the mining water tributary (MW). The first site (US) on the Nedvědička River is approximately 200 m upstream from the tributary. The second site (DS1) is approximately 200 m downstream from the tributary, and the third site (DS2) is located 800 m downstream from the tributary. The water quality was better in the tributary than in the river (xenologosaprobic character, high quality-acc. Water Framework Directive EU). The various physico-chemical characteristics of the water differ between the mining tributary and the Nedvědička River. These include higher water temperature (*e.g.*, up to 20 °C in winter) and conductivity (up to 1500 µS.cm⁻¹) as well as the amount of inorganic carbon, pH and lower level of organic pollution and the amount of oxygen in the mining water tributary compared with the Nedvědička River. Higher conductivity in the tributary was caused by an increased amount of SO₄²⁻ ions (690 mg.L⁻¹). Uranium concentration in the Nedvědička River is low and well below the limit required by Directive 2008/105/EC of the European Parliament (Hudcová *et al.*, 2012). More information about sampling sites has been reported in Růžičková *et al.* (2014).

Invertebrate and algal sampling

Samples of macroinvertebrates and algae were taken once a month from March 2008 to June 2009 (only January 2009 was omitted because of a large amount of snow) at each of the four sampling sites. Epilithon were sampled from riffle stones using the methodology according to CEN/TC 230N0540 (Guidance standard

for the surveying, sampling and laboratory analysis of phytobenthos in shallow running water). Cell density was estimated using a Sedgwick–Rafter chamber and was expressed as the number of cells in 1 mL (cell.mL^{-1}). For taxonomic analysis of diatoms, the sample was oxidized with hydrogen peroxide, and the reagents extracted by successive washing steps by centrifugation. Invertebrate samples were collected using a Hess sampler with a mesh size of 250 μm and a surface area of 0.08 m^2 . Two riffles and two pools were chosen at each sampling site. Two samples from the same habitat were pooled at each sampling site, thus we have two samples from each sampling site per month. In total, we obtained 120 samples. The collected material was fixed with 4% formaldehyde in the field, and macroinvertebrates were identified. Invertebrate taxa were assigned to functional feeding groups by reference to Moog (1995) and Cummins and Klug (1979) (Appendix A). Taxa that graze (scrape) and/or collect particles from the stream-bed surface were classified as scraper-collectors (SC) and taxa that chew and/or mine large particles from detritus were classified as shredders (S) (Ledger and Hildrew, 2005). Taxa that filter particles (with an algal component) from the stream were classified as filter-feeders (FF). Subfamilies of Chironominae, Orthocladiinae and Tanytarsinae were not assigned to functional feeding groups, because of the different feeding strategies of particular species within these groups.

Environmental data

For each sampling site, one value for each for conductivity, pH, amount of dissolved oxygen and oxygen saturation was measured during each monthly sampling in the field. Shading was estimated by the same person at each locality during each monthly sampling. The content of NH_4^+ , NO_3^- , PO_4^{3-} and SO_4^{2-} ions and TN (total amount of nitrogen), TC (total amount of carbon), IC (total amount of inorganic carbon) and TOC (total amount of organic carbon) were measured from each water sample in the laboratory. We monitored water temperature (by submersed electronic thermometer – ONEST[®] data logger set) at each sampling sites every 15 min. To evaluate the hydrodynamic characteristics of the sampling sites, the Froude number (Fr) was calculated using $Fr = U/\sqrt{gD}$ (D : depth, g : acceleration due to gravity, and U : current velocity at a depth of 0.4). The phi value (Hynes, 1970) was used to evaluate substrate coarseness.

Data analysis

The difference in environmental variables among the specific sampling sites was tested using the Student's paired t -test. Because the samples had a temporal dependence, we used the generalized estimating equations (GEE) with autoregressive (AR1) structure and Poisson

distribution to evaluate difference in abundance and taxa richness of macroinvertebrates and epilithon among the sampling sites. The model was: number of taxa (or abundance) \sim locality/habitat, habitat was nested in locality. The same method was used to test differences in abundance of particular feeding groups among the sampling sites. PERMANOVA was used to evaluate differences in community composition. Bray–Curtis dissimilarity and 499 permutations were used; permutations were constrained within locality (among sampling dates). The model was: community \sim locality/habitat*month, habitat was nested in locality. For epilithon only, the “locality*month” model was used, because it was sampled from only one habitat (riffles). Non-metric multidimensional scaling (NMDS; Kruskal, 1964) was used to visualize the main gradient in a species data set. MW is not included in NMDS because differences in abundance and taxa composition between MW and the Nedvědička River would cover differences between US and DS1 and DS2, which we were primarily focused on. Species abundance data were $\log(x + 1)$ transformed. Species data from different habitats (pools and riffles) were pooled, thus only one value per sampling is shown (60 samples) in the NMDS diagram. Pooling of species data was used because we had only one value for most measured environmental variables per sampling (see above) and environmental data were fitted linearly into the two-dimensional space of the NMDS plot. Only significant variables (based on 999 permutations) are shown in the NMDS diagram. The collected temperature data were scaled by day and month by means of these values and were used in the NMDS. For phi and Froude number, which were measured for riffles and pools separately, mean values of these parameters were fitted.

All analyses were performed in R (version 2.12.0; R Development Core Team, 2010) using the “vegan” (Oksanen *et al.*, 2011) and “geepack” (Hojsgaard *et al.*, 2006) packages.

Results

Environmental variables

The unaffected upstream site (US) differed significantly ($P < 0.05$) from the mining water inflow (MW) by having a higher amount of O_2 ($t = 5.1612$), NO_3^- ($t = 5.2562$), PO_4^{3-} ($t = 4.3036$), total nitrogen ($t = 6.4854$) and a lower value of pH ($t = -6.3722$), conductivity ($t = -22.239$), mean ($t = -9.4119$), minimum ($t = -8.8145$) and maximum ($t = -8.2843$) monthly temperature, total ($t = -2.7531$) and inorganic ($t = -7.1674$) carbon and phi ($t = -5.2925$).

We found a significant difference in physico-chemical variables between upstream (US) and downstream () sampling sites. US and DS sites differed significantly ($P < 0.05$) in minimum ($t = -14.962$ (DS1), $t = -2.9512$ (DS2)), mean ($t = -14.033$ (DS1), $t = -12.054$ (DS2)) and maximum ($t = -12.928$ (DS1), $t = -9.652$ (DS2))

Table 1. Minimum, maximum and median values of the measured environmental variables at sampling sites.

Site	US			MW			DS1			DS2		
	Min	Median	Max	Min	Median	Max	Min	Median	Max	Min	Median	Max
Variable												
Conductivity ($\mu\text{s.cm}^{-1}$)	202.2	318	410	987	1395	1580	400	604	930	415	583	789
pH	7.3	7.6	8.4	7.8	8.2	8.9	7.5	8.0	8.5	7.2	8.0	8.8
Dissolved O ₂ (mg.L ⁻¹)	8.7	11.4	12.7	7.6	8.8	10.1	8.1	10.2	12.3	8.2	10.3	12.0
O ₂ saturation (%)	86	96	118	78	98	116	81	98	120	78	97	119
Mean monthly temperature (°C)	2.9	11.7	15.4	12.6	17.9	19.9	4.8	13.4	16.4	4.8	13.3	16.3
Min. monthly temperature (°C)	0.1	7.4	13.3	8.0	13.5	17.2	1.9	9.1	15.0	1.7	9.1	14.8
Max. monthly temperature (°C)	5.8	15.0	19.0	18.1	21.2	24.1	7.4	16.8	20.6	7.4	16.3	20.7
NH ₄ ⁺ (mg.L ⁻¹)	0.4	0.7	1.6	0.0	0.6	1.6	0.0	0.5	1.1	0.0	0.5	1.3
NO ₃ ⁻ (mg.L ⁻¹)	8.6	29.5	41.9	0.4	12.9	30.5	13.8	22.0	41.1	13.6	21.3	41.5
PO ₄ ³⁻ (mg.L ⁻¹)	0.0	0.1	0.4	0.0	0.0	0.1	0.0	0.1	0.5	0.0	0.1	0.3
SO ₄ ²⁻ (mg.L ⁻¹)	44	48	66	430	500	690	80	170	280	81	145	270
TN (mg.L ⁻¹)	0.6	7.8	15.5	0.2	1.7	8.4	0.2	6.0	14.6	0.8	5.7	14.6
TC (mg.L ⁻¹)	11.8	21.3	64.8	16.9	32.8	63.9	12.9	20.5	38.9	12.7	20.5	37.9
IC (mg.L ⁻¹)	4.4	11.3	23.2	11.2	23.6	46.1	7.6	13.3	27.8	6.4	15.9	27.3
TOC (mg.L ⁻¹)	3.0	8.7	55.4	0.6	6.6	19.4	1.1	8.2	12.9	1.1	7.8	20.3
Phi value (riffles)	-7.0	-4.6	-1.7	-7.0	-3.4	-1.7	-7.4	-5.8	-3.2	-7.0	-4.4	-1.3
Phi value (pools)	-4.4	-0.7	2.4	-4.2	1.4	5.1	-5.1	-1.8	2.0	-4.5	-1.5	4.5
FROUDE number (riffles)	0.17	0.36	0.70	0.17	0.38	0.70	0.06	0.44	0.89	0.06	0.26	0.51
Froude number (pools)	0.01	0.08	0.17	0.01	0.11	0.17	0.01	0.05	0.12	0.01	0.06	0.14

US, unaffected locality upstream from the tributary; MW, the mining water inflow; DS1, first influenced locality downstream from the tributary; DS2, second influenced locality downstream from the tributary; TN, total amount of nitrogen; TC, total amount of carbon; IC, amount of inorganic carbon; TOC, total amount of organic carbon (from Růžičková *et al.*, 2014).

monthly temperature, conductivity ($t = -8.6291$ (DS1), $t = -9.0998$ (DS2)), pH ($t = -2.7593$ (DS1), $t = -3.134$ (DS2)), inorganic carbon amount ($t = -4.4075$ (DS1), $t = -5.322$ (DS2)), amount of NO₃⁻ ($t = 2.9458$ (DS1), $t = 2.781$ (DS2)), TN ($t = 5.9897$ (DS1), $t = 5.5133$ (DS2)) and dissolved oxygen ($t = 2.4203$ (DS1), $t = 2.4862$ (DS2)). These variables also differed the most between the mining water tributary and US (see Table 1). We found no significant difference in case of shading. The recorded variables were very similar between DS1 and DS2, significant differences ($P < 0.05$) were found only in the case of phi values ($t = -2.4672$).

Epilithon communities

We found 76 taxa of algae and bacteria. The taxonomic richness of algae and bacteria was significantly lower at US than at DS1 (Wald = 9.88, $P < 0.05$); US did not differ from DS2. US did not differ significantly from DS1 or DS2 in the abundance of epilithon. The mean recorded densities (cell.mL⁻¹) were 11918 at US, 13703 at DS1 and 11093 at DS2.

US differed significantly from MW in the number of cells (Wald = 17.18, $P < 0.05$) as well as in the number of algae and bacteria taxa (Wald = 75.63, $P < 0.05$), density and diversity were much higher in MW.

The results of the PERMANOVA showed that sampling sites differed significantly in community composition (Table 2). DS1 had more mutual taxa with the mining water tributary community MW (69% of species occurred in both sites) than with US (40%) and even with DS2 (57%). On the other hand, the algal community of

DS2 was more similar to that of US (50 %) than to that of MW (42 %). Figure 1 shows the relative composition of epilithon at each locality. The graphical output clearly shows the differences between the US and DS sites and the similarity between DS1 and DS2. The main difference is caused by a decrease in Rhodophyta and an increase in Chlorophyta numbers at the affected sampling sites. For a list of algal taxa and their presence/absence at each sampling site, see Appendix B.

Abundance and taxa richness of macroinvertebrates

We found 45 taxa of benthic macroinvertebrates. The number of taxa was very similar among sampling sites on the Nedvědička River. We recorded 42 taxa at US, 42 at DS1, 40 at DS2 and 30 in MW. Significant differences occurred between US and DS2 (Wald = 6.13, $P < 0.05$) and between US and MW (Wald = 10.22, $P < 0.05$).

The abundance of herbivorous macroinvertebrates differed significantly among sampling sites ($\chi^2 = 11.4$, $P < 0.05$). The abundance of herbivorous macroinvertebrates differed significantly between US and DS1 (Wald = 13.765, $P < 0.05$) and between US and DS2 (Wald = 7.36, $P < 0.05$, see Fig. 2). The number of individuals was significantly higher in MW than in US at the Nedvědička River (Wald = 11.940, $P < 0.05$). In total, we found 5117 individuals at US, 4373 at DS1, 7542 at DS2 and 10046 at MW.

The abundance of Coleoptera was significantly higher in DS1 (Wald = 6.64, $P < 0.05$) and DS2 (Wald = 4.72, $P < 0.05$) when compared with US (see Fig. 3). The abundance of Gastropoda was significantly increased at

Table 2. Results of PERMANOVA on epilithon and herbivorous macroinvertebrate species data.

Epilithon	Among all sampling sites		US versus DS1		US versus DS2		DS1 versus DS2		MW versus US	
	d.f.	<i>F</i>	d.f.	<i>F</i>	d.f.	<i>F</i>	d.f.	<i>F</i>	d.f.	<i>F</i>
Locality	3	228.219*	1	33.942*	1	42.574*	1	48.002*	1	434.13*
Month	14	37.309*	14	3.415*	14	3.659*	14	18.895*	14	14.71*
Locality:month	40	5.919*	–	–	–	–	–	–	14	9.53*
Residuals	2		14		14		14		29	
Macroinvertebrates	Among all sampling sites		US versus DS1		US versus DS2		DS1 versus DS2		MW versus US	
Locality	3	7.624*	1	6.132*	1	5.661*	1	1.115 ns	1	19.254*
Month	14	2.081*	14	1.683*	14	1.958*	14	1.600*	14	1.771*
Locality:habitat	4	4.431*	2	4.383*	2	5.192*	2	4.212*	2	5.389*
Locality:month	–	–	–	–	–	–	–	–	14	1.428*
Residuals	98		42		42		42		28	

MW, the mining water inflow; US, unaffected locality upstream from the tributary; DS1, first influenced locality downstream from the tributary; DS2, second influenced locality downstream from the tributary; d.f., degrees of freedom; *F*, *F* statistic value; * $P < 0.05$; ns, not significant.

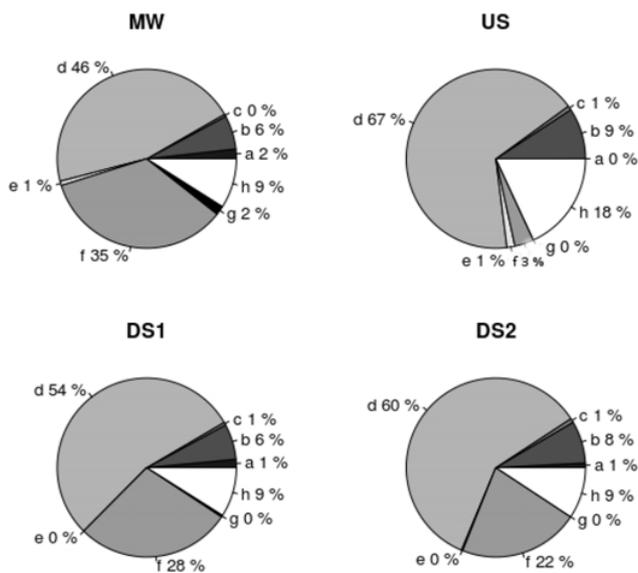


Fig. 1. Epilithon community distribution. a: Bacteria, b: Cyanobacteria, c: Xanthophyceae, d: Bacillariophyceae, e: Euglenophyta, f: Chlorophyta, g: Charophyta, h: Rhodophyta. MW, the mining water tributary; US, unaffected sampling site; DS1, first sampling site downstream from the tributary; DS2, second sampling site downstream from the tributary.

DS1 (Wald = 17.58, $P < 0.05$) and DS2 (Wald = 12.29, $P < 0.05$) compared with US. However, the increase in the abundance of Gastropoda downstream from the tributary is caused by an increase of one species of *Potamopyrgus antipodarum* (Gray) spreading out from MW. On the other hand, no effect on abundance was recorded in the case of Ephemeroptera and Diptera (Fig. 3). The abundance of Trichoptera decreased significantly (Wald = 9.49, $P < 0.05$) downstream from the tributary (at DS1) compared with US. The mining water tributary differed significantly from US in abundance of Coleoptera (Wald = 44.42, $P < 0.05$), Gastropoda (Wald = 46.65, $P < 0.05$), Ephemeroptera (Wald = 17.98, $P < 0.05$) and Diptera (Wald = 8.63, $P < 0.05$). For a list of species and their presence/absence at sampling sites, see Appendix A.

Functional feeding groups

We tested for differences among US (upstream site community) and DS1 and DS2 (downstream site communities) and found some statistically significant differences in abundance of particular feeding groups. The dominant functional group in US was FF, closely followed by SC, while S occurred in smaller numbers. Downstream, the most abundant group was SC. Increases in SC at downstream sites compared with US were statistically significant (Wald = 13.17 (DS1), Wald = 80.21 (DS2), $P < 0.05$). Numbers of FF decreased downstream. S numbers increased downstream, but they remained the smallest group. MW differed from US in the abundance of SC (Wald = 11.22, $P < 0.05$) and FF (Wald = 16.31, $P < 0.05$). The differences in the abundance of species within particular functional groups are shown in Figure 4. The taxonomic classification of functional feeding groups is shown in Appendix A.

Invertebrate community response

US significantly differed from DS1 and DS2 in community composition according to the results of PERMANOVA. More detailed results of PERMANOVA are listed in Table 2. Differences between unaffected (US) and affected (DS1 and DS2) communities are visible in the NMDS diagram; US is well separated from the DS1 and DS2 sites in a two-dimensional space (Fig. 5). The position of sampling sites can be explained with fitted variables. The higher values of temperature, conductivity and pH, as well as green algae density, are in the direction of DS sites; US is nutrient richer (NO_3^-).

Discussion

The mining water tributary altered the species composition and abundance of herbivorous macroinvertebrates in the Nedvědička River in two possible ways: (i) through a change in the physico-chemical properties of the water,

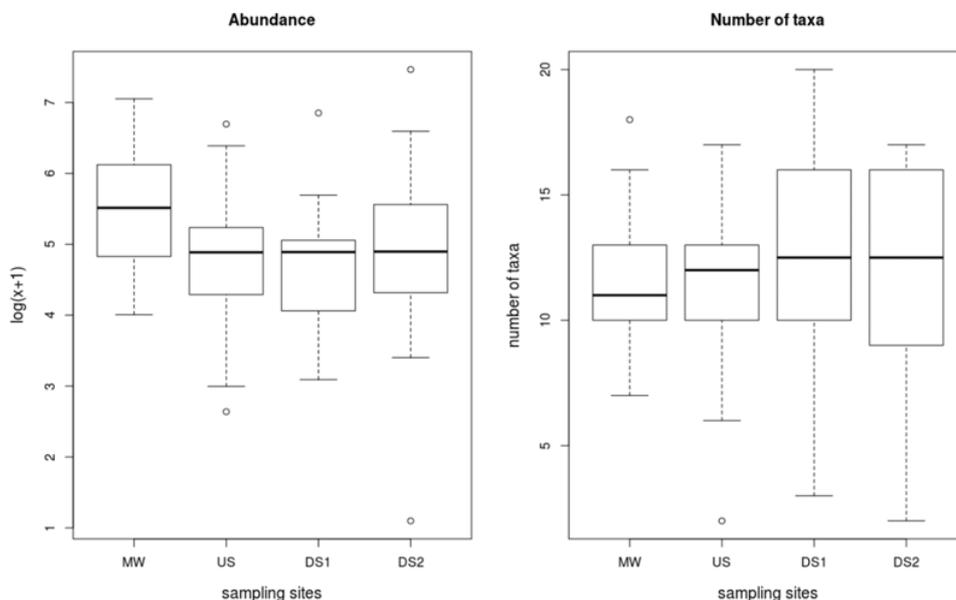


Fig. 2. Box-plots of abundance and taxa richness for three sampling sites. MW, the mining water tributary; US, unaffected sampling site; DS1, first sampling site downstream from the tributary; DS2, second sampling site downstream from the tributary.

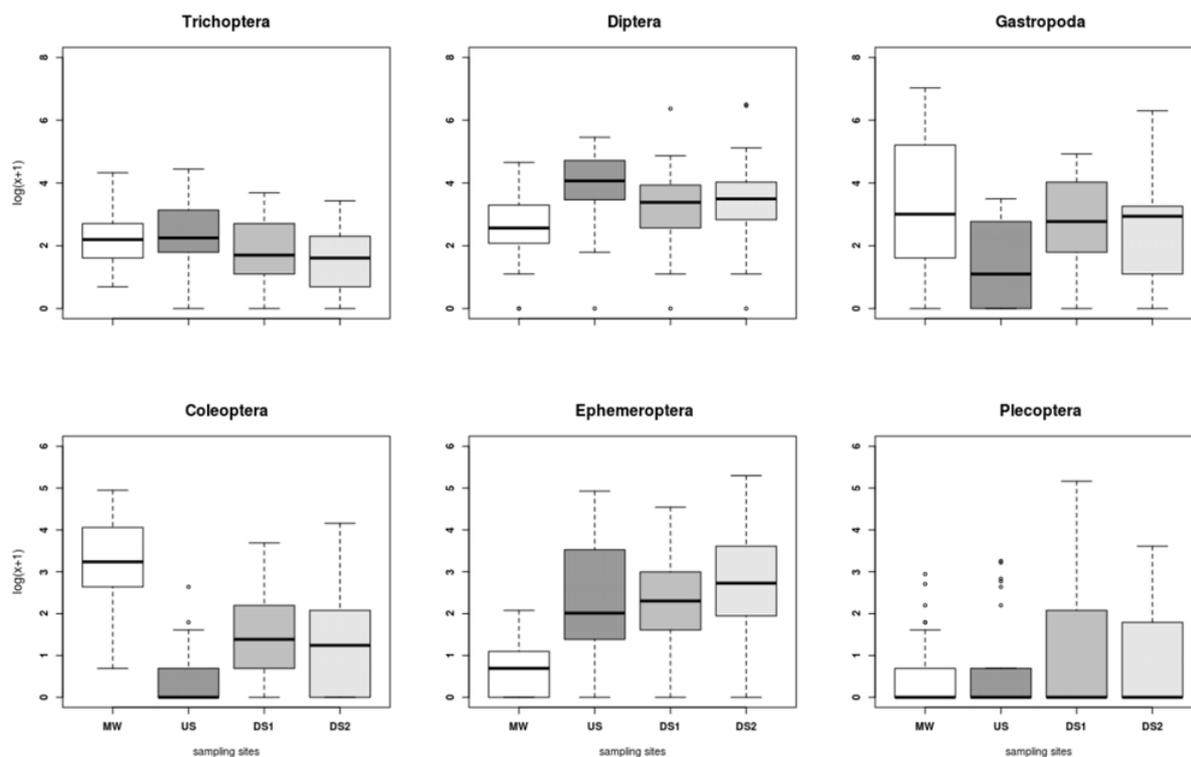


Fig. 3. Box-plots of particular taxa abundance for three sampling sites. MW, the mining water tributary; US, unaffected sampling site; DS1, first sampling site downstream from the tributary; DS2, second sampling site downstream from the tributary.

or (ii) through a change in food availability. However, the most likely scenario is a combination of the two working in concert. We also observed a shift in functional feeding group dominance from filter-feeders in US to scraper-collectors in both downstream sites.

Upstream and downstream sites differed in physico-chemical parameters, such as temperature, conductivity, inorganic carbon amount, pH (higher values downstream) and the amount of nitrogen and dissolved oxygen (lower values downstream); these changes were caused by the

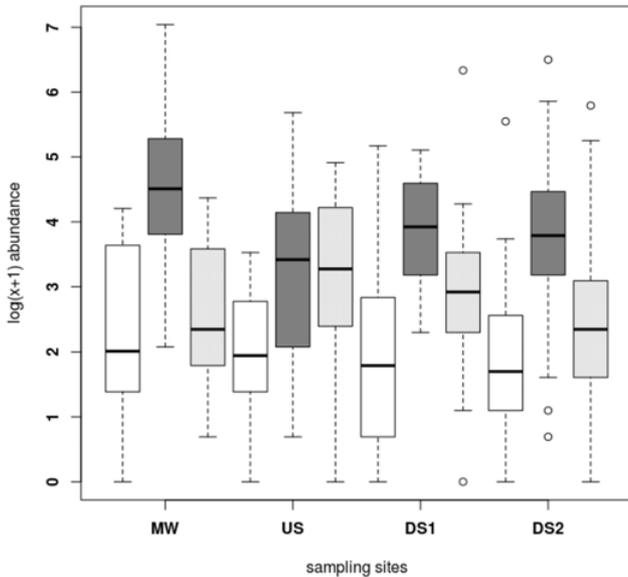


Fig. 4. Abundance of S, shredders (white); SC, scraper-collectors (dark grey) and F, filter-feeders (light grey) at sampling sites. MW, the mining water tributary; US, unaffected sampling site; DS1, first sampling site downstream from the tributary; DS2, second sampling site downstream from the tributary.

mining water tributary. A high conductivity level was caused by a large amount of sulphate ions in the mining water. The water downstream from the tributary was also poorer in nitrogen, compared with US. Nonetheless, 800 m downstream from the tributary (DS2), temperature and other environmental characteristics were still different from those at the upstream site (US) and more similar to those at DS1. The Nedvědička River did not return to its “natural state” in the monitored stretch; a return to a natural condition depends on many factors (*e.g.*, velocity, discharge, biotic conditions) and it can last several kilometres (see [Merovich and Petty, 2007](#)). We assume that water temperature and mineralization (represented by conductivity, IC, pH), alongside the abundance of epilithon, were likely responsible for the change in macroinvertebrate communities. This assumption was also confirmed when we fitted all environmental variables into the species data NMDS plot.

The total abundance of herbivorous macroinvertebrates decreased slightly immediately downstream from the tributary, then significantly increased again further downstream at DS2. This growth was mainly caused by a higher abundance of *Potamopyrgus antipodarum*, which occurred in large numbers in MW, DS1 and especially at DS2. This species was completely absent from the upstream site US, probably due to river freezing during the winter, which is limiting factor for this species ([Quinn *et al.*, 1994](#); [Richards *et al.*, 2001](#)). The abundance of Orthocladiinae dropped at DS1 relative to the US and rose again further downstream at site DS2. Orthocladiinae are considered cool stream inhabitants and their negative response to temperature is known ([Hogg and Williams, 1996](#); [Worthington *et al.*, 2015](#)). Some taxa, such as

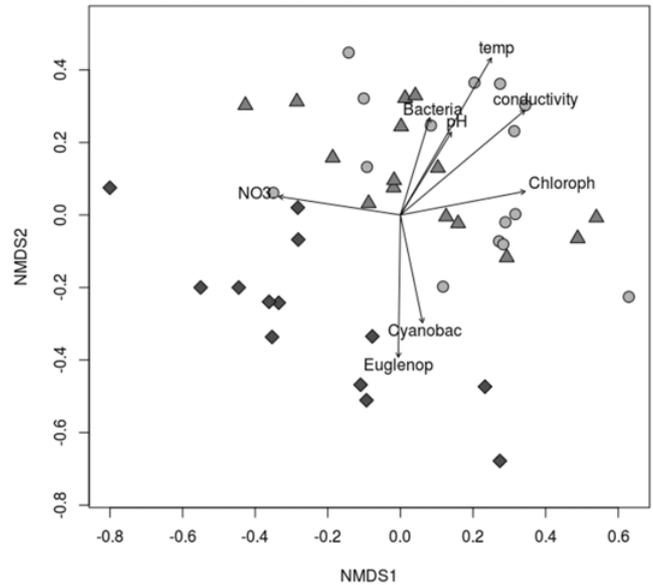


Fig. 5. NMDS diagram. Bray–Curtis dissimilarity, 2D stress, 0.15; diamond, US; triangle, DS1; circle, DS2. Black arrows represent fitted variables. Only significant variables are displayed ($P < 0.05$, 999 permutations).

Coleoptera (*Limnius volckmari* (Panzer), *Elmis maugetii* and *Hydraena gracilis* Germar) and Plecoptera (*Leuctra* sp.), increased their abundance immediately downstream. A positive relation between *Limnius volckmari* and warmer water was also confirmed by [Haidekker and Hering \(2008\)](#) and [Worthington *et al.* \(2015\)](#). The largest decrease in abundance we observed was with Trichoptera, especially *Hydropsyche* sp. We observed a shift in community composition between US and both DS sites, whereas DS1 and DS2 were very similar in species composition. Community composition changes frequently occur under thermal pollution, some sensitive species are replaced by more tolerant taxa (*e.g.*, [Wellborn and Robinson, 1996](#); [Živić *et al.*, 2013](#)), EPT taxa usually decrease and non-insect taxa experienced a rising abundance.

The effect of human-driven influences on the functional characteristics of aquatic macroinvertebrates communities were recorded previously (*e.g.*, [Wilkerson *et al.*, 2010](#); [Kratina *et al.*, 2012](#); [Fu *et al.*, 2015](#)). We expected an increase of scrapers downstream of the heated mining water tributary because of elevated amounts of epilithon under warmer temperatures ([Poff and Matthews, 1986](#); [Wilkerson *et al.*, 2010](#)). SC became the dominant functional feeding group downstream from the tributary, whereas US was dominated by FF. The number of FF could increase in warmer water according to previous studies ([Poff and Matthews, 1986](#); [Friberg *et al.*, 2009](#)) and this increase could be explained by accelerated litter decomposition. We found larger numbers of black flies (Simuliidae) downstream of the tributary but the total number of FF did not rise due to a drop in the numbers of net-building caddisflies.

The negative effect of high conductivity on macroinvertebrate abundance and especially taxa richness is well

documented (*e.g.*, Dumnicka and Galas, 2006; Pond *et al.*, 2008; Timpano *et al.*, 2015). Although we did not record a decrease in taxa richness in DS1 (and only a small difference in DS2), the decrease of some taxa abundances could be explained by higher ion concentration. We assumed pH and oxygen had a lower effect on taxa abundance (see Quinn and Hickey, 1990). Differences between DS1 and DS2 in abundance of particular taxa (*e.g.*, *Potamopyrgus antipodarum*) can be easily explained by coarser substratum in DS1 (against DS2).

We observed an increase in the richness of algal taxa immediately downstream of the tributary. The DS1 community was enriched by species from MW and the species composition of these two sites was very similar. An increase in the number of taxa under small temperature increases is known (*e.g.*, Talmage and Coutant, 1979; Descy and Mouvet, 1984). The diatom community seems to be most sensitive to environmental changes. Here we observed the biggest change in species composition and species-specific response to environmental variables. The replacement of sensitive diatom taxa by generalists was also observed in previous studies (Descy and Mouvet, 1984; Piggott *et al.*, 2015). We also found an increase in the number of green algae, which usually favour higher temperatures (Descy and Mouvet, 1984). A decrease in red algae (mainly of the genus *Batrachospermum*) was recorded in the Nedvědička River; a negative correlation between temperature and freshwater Rhodophyta was reported previously by Sheath (1984) or Leukart and Hanelt (1995).

The amount of algae was greatest in the mining water tributary (MW). Density of epilithon was bigger downstream (DS1) than upstream (US) from the tributary. However, the differences were not statistically significant. Only a small increase of epilithon at DS1 versus US could be explained by a small and insufficient difference in temperature (1.8 °C). Another possible explanation is that the riparian canopy along a monitored stretch of the Nedvědička River enabled only limited algae growth. Poor epilithon response could be explained by the increasing amount of SC downstream from the tributary (see Friberg *et al.*, 2009). An increased amount of epilithon consumers could indicate that there are larger quantities of algae available to consumers with increasing temperature. The lack of a relationship between epilithon and temperature suggests that grazing rates, and consequently algal productivity, are higher in the warmer part of the Nedvědička River.

In conclusion, we observed a statistically significant increase in epilithon taxa numbers and in community composition, but only a small increase in density. A decrease in algal abundance at the further downstream site (DS2) could be explained by higher herbivore abundance. We cannot say that the mining water tributary had a positive or negative effect on herbivorous macroinvertebrate communities, but it has clearly changed their composition. There were winners and losers: species with broader ecological valence, such as *Potamopyrgus antipodarum*, made use of the opportunity and profited,

while *Ancylus fluviatilis* (O. F. Müller) and Orthocladinae showed reduced numbers. Different species were probably affected by different environmental influences (mineralization, temperature, biotic interactions) and their combination. More detailed research focused on autecology and/or experimental separation of temperature and mineralization effects on water biota would be helpful to better understand the mining water effect.

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Appendix

Appendix A. List of macroinvertebrate taxa found at particular sampling sites, their abbreviations and functional feeding group assignment.

Taxon	FFG	MW	US	DS1	DS2
Ephemeroptera					
<i>Baetis rhodani</i> (Pictet, 1845)	SC	+	+	+	+
<i>Caenis pseudorivulorum</i> (Keffermüller, 1960)	SC	+	+	+	
<i>Ecdyonurus torrentis</i> (Kimmins, 1942)	SC		+	+	+
<i>Ecdyonurus</i> sp.	SC		+	+	
<i>Ephemera danica</i> (Müller, 1764)	SC		+	+	+
<i>Ephemerella ignita</i> (Poda, 1761)	SC		+	+	+
<i>Paraleptophlebia submarginata</i> (Stephens, 1835)	SC		+		
<i>Rhithrogena</i> sp.	SC		+	+	+
Trichoptera					
<i>Anabolia furcata</i> (Brauer, 1857)	S		+		
<i>Athripsodes</i> sp.	S			+	
<i>Halesus digitatus</i> (Schrank, 1781)	S		+	+	+
<i>Hydropsyche instabilis</i> (Curtis, 1834)	F	+	+	+	+
<i>Hydropsyche pellucidula</i> (Curtis, 1834)	F	+	+	+	+
<i>Hydropsyche saxonica</i> (McLachlan, 1884)	F	+	+	+	
<i>Hydropsyche siltalai</i> (Döhler, 1964)	F	+	+	+	+
<i>Hydropsyche</i> sp. juv.	F	+	+	+	+
<i>Hydroptila vectis</i> (Curtis, 1834)	SC	+			+
<i>Chaetopteryx</i> sp.	S		+	+	+
<i>Lepidostoma hirtum</i> (Fabricius, 1775)	S		+		
Leptoceridae g. sp. juv.	S		+	+	+
Limnephilinae sp. Juv	S	+	+	+	+
<i>Potamophylax luctuosus</i> (Piller and Mitterpacher, 1783)	S			+	+
<i>Psychomyia pusilla</i> (Fabricius, 1781)	SC			+	
Psychomyidae g. sp. juv.	SC		+		
<i>Silo pallipes</i> (Fabricius, 1781)	SC	+	+	+	+
<i>Tinodes unicolor</i> (Pictet, 1834)	SC	+			
<i>Wormaldia occipitalis</i> (Pictet, 1834)	F	+			
Plecoptera					
<i>Leuctra digitata</i> (Kempny, 1899)	S		+		
<i>Leuctra fusca</i> (Linnaeus, 1758)	S		+	+	+
<i>Leuctra</i> sp.	S	+	+	+	+
Gastropoda					
<i>Ancylus fluviatilis</i> (O. F. Müller, 1774)	SC	+	+	+	+
<i>Potamopyrgus antipodarum</i> (Gray, 1843)	SC	+		+	+
Diptera					
<i>Brillia modesta</i> (Kieffer, 1909)	S	+	+	+	+
<i>Antocha</i> sp.	SC		+	+	+
<i>Berdeniella</i> sp.	SC		+	+	+
<i>Chironominae</i>		+	+	+	+
<i>Orthocladinae</i>		+	+	+	+
<i>Prodiamesa olivacea</i> (Meigen, 1818)	F	+	+	+	+
Simuliidae g. sp.	F	+	+	+	+
Tanypodinae g. sp.		+	+	+	+
Tanytarsini	F	+	+	+	+
<i>Tipula</i> sp.	S		+	+	+
<i>Tipula lateralis</i> (Meigen, 1804)	S	+	+	+	+
<i>Tipula vittata</i> (Meigen, 1804)	S		+		
<i>Tvetenia</i> sp.	SC	+	+	+	+
Coleoptera					
<i>Elmis maugetii</i> (Latreille, 1798)	SC	+	+	+	+
<i>Halipus</i> sp.	S	+			
<i>Hydraena gracilis</i> (Germer, 1824)	SC	+	+	+	+
<i>Limnius volckmari</i> (Panzer, 1793)	SC	+	+	+	+
<i>Oulimnius tuberculatus</i> (P.W. and J. Müller, 1806)	SC	+		+	+
Crustacea: Amphipoda					
<i>Gammarus fossarum</i> (Koch in Panzer, 1835)	S	+	+	+	+

FFG, functional feeding group; MW, mining water tributary; US, unaffected upstream site; DS1, first downstream site; DS2, second downstream site; SC, scraper-collector; S, shredder; F, filtrator.

Appendix B. List of bacteria and algae found at particular sampling sites.

Taxon	MW	US	DS1	DS2
Bacteria				
<i>Beggiatoa alba</i> (Vaucher) Trevisan	+	+	+	+
<i>Clonothrix fusca</i> (Roze, 1896)	+		+	+
<i>Thiothrix nivea</i> (Rabenhorst) Winogradsky	+		+	+
Cyanobacteria				
<i>Aphanocapsa rivularis</i> (Carmichael) (Rabenhorst, 1865)	+	+	+	+
<i>Chamaesiphon incrustans</i> (Grunow, 1865)	+		+	+
<i>Chamaesiphon subglobosus</i> (Rostafinski) (Lemmermann, 1910)		+	+	+
<i>Heteroleibleinia kuetzingii</i> (Schmidle) (Compère, 1985)	+	+	+	+
<i>Homoeothrix varians</i> (Geitler, 1927)		+	+	+
<i>Leptolyngbya foveolarum</i> (Montagne ex Gomont) (Anagnostidis and Komárek, 1988)	+		+	+
<i>Phormidium fonticolum</i> Kützing ex Gomont	+	+	+	+
<i>Tapinothrix bornetii</i> (Sauvageau, 1892)		+	+	+
Xanthophyceae				
<i>Tribonema</i> sp.	+	+	+	+
<i>Vaucheria</i> sp.	+		+	+
Bacillariophyceae				
<i>Achnanthes lanceolata</i> (Brébisson ex Kützing) (Grunow, 1880)	+		+	+
<i>Achnanthes minutissima</i> (Kützing, 1833)	+	+	+	+
<i>Amphora pediculus</i> Grunow ex A.Schmidt	+		+	+
<i>Aulacoseira granulata</i> (Ehrenberg) (Simonsen, 1979)		+		
<i>Caloneis amphibaena</i> (Bory de Saint Vincent) (Cleve, 1894)	+	+		
<i>Caloneis silicula</i> (Ehrenberg) (Cleve, 1894)	+			
<i>Cocconeis pediculus</i> (Ehrenberg, 1838)	+	+	+	+
<i>Cocconeis placentula</i> (Ehrenberg, 1838)	+		+	
<i>Cymatopleura solea</i> (Brébisson) (W. Smith, 1851)		+		
<i>Cymbella naviculiformis</i> (Auerswald ex Heiberg 1863)		+		+
<i>Cymbella ventricosa</i> (C. Agardh 1830)			+	+
<i>Diatoma elongatum</i> (Lyngbye) (C. Agardh, 1824)	+		+	+
<i>Diatoma vulgare</i> (Bory de Saint-Vincent, 1824)	+	+	+	+
<i>Eunotia bilunata</i> (Ehrenberg) (Schaarschmidt, 1880)		+	+	
<i>Eunotia tenella</i> (Grunow) (Hustedt, 1913)		+		
<i>Fragillaria virescens</i> (Ralfs, 1843)		+		
<i>Frustulia vulgare</i> (Thwaites) (De Toni, 1891)		+		
<i>Gomphonema angustatum</i> (Kützing) (Rabenhorst, 1864)			+	
<i>Gomphonema dichotomum</i> (Kützing, 1833)			+	
<i>Gomphonema parvulum</i> (Kützing) (Kützing, 1849)	+	+	+	+
<i>Gomphonema montanum</i> (Schumann, 1867)		+		
<i>Gyrosigma acuminatum</i> (Kützing) (Rabenhorst, 1853)		+		
<i>Gyrosigma spenceri</i> (W. Smith) (Griffith and Henfrey, 1856)	+			
<i>Melosira varians</i> (C. Agardh, 1827)	+	+	+	+
<i>Meridion circulare</i> (Greville) (C. Agardh, 1831)	+	+	+	+
<i>Navicula avenacea</i> (Rabenhorst) (Brébisson ex Grunow, 1878)	+	+	+	+
<i>Navicula cincta</i> (Ehrenberg) (Ralfs, 1861)	+			
<i>Navicula cryptocephala</i> (Kützing, 1844)	+	+	+	+
<i>Navicula rhynchocephala</i> (Kützing, 1844)		+	+	+
<i>Navicula salina</i> (W. Smith) (Schütt, 1896)	+		+	
<i>Navicula salinarum</i> (Grunow, 1880)	+		+	
<i>Navicula slesvicensis</i> (Grunow, 1880)	+		+	
<i>Nitzschia denticula</i> (Grunow, 1880)	+			
<i>Nitzschia filiformis</i> (W. Smith) Hustedt				+
<i>Nitzschia commutata</i> (Grunow, 1880)				+
<i>Nitzschia gracilis</i> (Hantzsch, 1860)	+		+	
<i>Nitzschia linearis</i> (C. Agardh) (W. Smith, 1853)	+	+	+	
<i>Nitzschia sigma</i> (Kützing) (W. Smith, 1853)	+		+	
<i>Nitzschia sigmoidea</i> (Nitzsch) (W. Smith, 1853)	+		+	
<i>Nitzschia obtusa</i> (W. Smith, 1853)	+			
<i>Nitzschia palea</i> (Kützing) (W. Smith, 1856)	+		+	
<i>Pinnularia apendiculata</i> (C. Agardh) (Cleve, 1895)				+
<i>Pinnularia major</i> (Kützing) (Rabenhorst, 1853)		+		
<i>Pinnularia viridis</i> (Nitzsch) (Ehrenberg, 1843)		+		

Appendix B. (Contd.)

Taxon	MW	US	DS1	DS2
<i>Rhoicosphaenia curvata</i> (Kützing) (Grunow, 1860)	+			
<i>Surirella bifrons</i> Ehrenberg				+
<i>Surirella ovata</i> (Kützing, 1844)	+	+	+	+
<i>Surirella ovalis</i> (Brébisson, 1838)	+			
<i>Synedra rumpens</i> (Kützing, 1844)		+		+
<i>Synedra tabulata</i> (C. Agardh) (Kützing, 1844)	+		+	
<i>Synedra ulna</i> (Nitzsch) (Ehrenberg, 1832)	+	+	+	+
Euglenophyta				
<i>Euglena acus</i> (O.F. Müller) (Ehrenberg, 1830)	+		+	
<i>Phacus brevicauda</i> Lemmermann	+			
<i>Trachelomonas hispida</i> (Perty) (F. Stein, 1878)	+			
<i>Trachelomonas volvocina</i> (Ehrenberg) (Ehrenberg, 1834)	+		+	
Chlorophyta				
<i>Cladophora glomerata</i> (Linnaeus) (Kützing, 1843)	+	+	+	+
<i>Oedogonium capillare</i> (Kützing ex Hirn, 1900)	+	+	+	+
<i>Pediastrum tetras</i> (Ehrenberg) (Ralfs 1845)	+		+	
<i>Scenedesmus obliquus</i> (Turpin) (Kützing, 1833)	+		+	
Charophyta				
<i>Closterium litorale</i> (F. Gay, 1884)	+		+	
<i>Klebsormidium flaccidum</i> (Kützing) (P.C. Silva, K.R. Mattox and W.H. Blackwell, 1972)	+			
Rhodophyta				
<i>Batrachospermum</i> sp.	+	+	+	+
<i>Chantransia</i> sp.	+	+	+	+

MW, mining water tributary; US, upstream site; DS1, first downstream site; DS, second downstream site.