Environmental Pollution xxx (2016) xxx-xxx

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com

Effects of potash mining on river ecosystems: An experimental study☆

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ARTICLE INFO

Article history:
Received 11 October 2016
Received in revised form 23 December 2016
Accepted 25 December 2016
Available online xxx

Keywords:
Salinization
Salt pulses
Mining
Diatoms
Aquatic invertebrates
Biotic indices

ABSTRACT

In spite of being a widespread activity causing the salinization of rivers worldwide, the impact of potash mining on river ecosystems is poorly understood. Here we used a mesocosm approach to test the effects of a salt effluent coming from a potash mine on algal and aquatic invertebrate communities at different concentrations and release modes (i.e. press versus pulse releases). Algal biomass was higher in salt treatments than in control (i.e. river water), with an increase in salt-tolerant diatom species. Salt addition had an effect on invertebrate community composition that was mainly related with changes in the abundance of certain taxa. Short (i.e. 48 h long) salt pulses had no significant effect on the algal and invertebrate communities. The biotic indices showed a weak response to treatment, with only the treatment with the highest salt concentration causing a consistent (i.e. according to all indices) reduction in the ecological quality of the streams and only by the end of the study. Overall, the treatment's effects were time-dependent, being more clear by the end of the study. Our results suggest that potash mining has the potential to significantly alter biological communities of surrounding rivers and streams, and that specific biotic indices to detect salt pollution should be developed.

1. Introduction

Resource extraction is increasing worldwide to meet human demands for energy and goods (Krausmann et al., 2009; Reichl et al., 2016). In 2014 the world production of mineral raw materials was of 17,434 million metric tons, providing a total revenue of thousands of billions of US dollars (Reichl et al., 2016). One valuable mineral is potash, with a world production of 39.55 million metric tons in 2014, increasing at a rate of 19.52% from 2010 (Reichl et al., 2016). Many mining operations generate wastes (e.g. potash mines can generate tailings dominated by NaCl) that are one of the world's largest chronic waste concerns (Bian et al., 2012). It is estimated that hundreds of thousands of tons of mine tailings are produced per day (Jakubick et al., 2003). For example, in the US mining activities generate 10 times as much solid waste as municipal solid waste per capita (Hudson-Edwards et al., 2011). Very often these wastes are stored in impoundments around the mines, from where they can reach surface waters by seepage through embankments or through the base of the tailings pile (Hudson-Edwards et al., 2011).

The ecological impact of coal and metal mines on surface waters has received considerable attention from the scientific community (Dudka and Adriano, 1997; Palmer et al., 2010). Several studies have showed that biological communities can be seriously impaired by mine wastes (e.g. Clements et al., 2000; Pond et al., 2014). However, the potential effects of potash mining on river ecosystems are less understood (Bäthe and Coring, 2011 ; Braukmann and Böhme, 2011; Cañedo Argüelles et al., 2012; Coring and Bäthe, 2011; Schulz, 2016; Ziemann et al., 2001). The potassium in potash is one of the most important components of commercial soluble fertilizers, since it is an essential plant nutrient. Annual potash production capacity was projected to increase globally from 52 million tons in 2015 to 61 million tons in 2019, and world consumption for all uses of potash was projected to increase gradually from 35.5 to 39.5 million tons for the same period (Ober, 2016). As other mining activities, potash extraction generates large quantities of waste. For example, in the potash mines of central Catalonia, 3 tons of waste are generated for each ton of potash that is extracted (Gorostiza Langa, 2014). These wastes are mainly composed of NaCl and they are often stored in open locations near the mines, resulting in artificial mountains (i.e. mine tailings). Although management measures such as brine collectors have been implemented (Martín-Alonso, 1994), the salts from the tailings are still dissolved by rain and humidity (Cañedo Argüelles et al., 2012; Otero and Soler, 2002) and they often leak from the collecting and retention infrastructures (Gorostiza Langa, 2014). Thus, large quantities of these salts end up in streams and rivers around the potash mining areas.

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

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http://dx.doi.org/10.1016/j.envpol.2016.12.072
0269-7491/

Since river organisms are adapted to freshwater, the increase in the salt concentration caused by potash mining wastes has the potential to significantly alter the river ecosystem (Cañedo-Argüelles et al., 2013). Studies on the River Werra (Germany), which was heavily impacted by potash mining, showed a recovery in biological communities after salt pollution was lowered from maximum chloride concentrations of 27 g L^{-1} in 1992 to 2.5 g L^{-1} in 2000 due to the implementation of management practices (Bäthe and Coring, 2011a, 2011b; Coring and Bäthe, 2011). These studies suggested that biological quality could be further improved if maximum chloride concentration was lowered to 1.5 g L^{-1}. However, the information on the ecological impacts of freshwater salinization is still too scarce to robustly guide management decisions and there are important questions that remain unanswered. Moreover, in field studies it is difficult to isolate the effect of salt pollution on aquatic organisms from the effect of other variables (e.g. habitat characteristics, nutrient concentrations). In this regard, mesocosm studies allow conducting experiments under controlled conditions and, at the same time, capturing some of the complexity of natural ecosystems (Odum, 1984). Previous mesocosm studies on the potential effects of potash mining pollution on river ecosystems suggested that high salt concentrations (i.e. higher than 3 g L^{-1}) could lead to significant changes in the aquatic macroinvertebrate communities (Cañedo-Argüelles et al., 2015, 2012), and that short salt pulses had little effect on diatom and invertebrate communities (Cañedo Argüelles et al., 2014). In all these mesocosm studies the changes in community composition were related with changes in abundance of the different taxa, but not with changes in species richness. However, these studies had two important limitations: they were conducted over short periods of time (i.e. maximum duration = 16 days) and they used NaCl as a proxy to potash mine wastes.

Here we used a mesocosm approach to study the potential impact of potash mining on river ecosystems and to provide management recommendations. Although different strategies exist to avoid disposing salts into rivers and streams (Martín-Alonso, 1994), in some cases salt disposal might be unavoidable. Thus, one of the most pressing management concerns is to know what is the best disposal strategy: to dispose salts continuously at low concentrations (i.e. press release) or to dispose salts at higher concentrations during short periods of time (i.e. pulse releases). Also, there is a need to know what is the maximum salt concentration that should be allowed in rivers and streams to prevent damaging the ecosystem and degrading its ecological status. Here, we tested the effects of a salt effluent coming from a potash mining tailing heap in the River Werra basin (Germany) on algal and aquatic invertebrate communities at different concentrations (similar to those currently registered in the River Werra, impacted by potash mining) and different salt disposal schemes (i.e. pulse versus press releases). We focused on algae and invertebrates, which are good indicators of water quality (Potapova and Charles, 2007; Rosenberg and Resh, 1993). Our initial hypothesis was that high salt concentrations (i.e. above 3 g L^{-1}) would have significant effects on algal and invertebrate communities, whereas moderate and low salt concentrations (i.e. below 3 g L^{-1}) would not. We also expected taxa richness to be unaffected by the salt treatment, leading to a weak response of biotic indices (which heavily rely on richness as an indicator). Finally, we expected short salt pulses (i.e. 48 h long) to have no significant effect on the algal and invertebrate communities due to the capacity of organisms to tolerate these short phases of stress and to recover between pulses (Cañedo Argüelles et al., 2014).

2. Methods

2.1. Experimental setup

The experiment was performed in a set of 12 artificial streams (Supplementary material) of 3 m of length, fed by river water coming from the upper part of the Ter River (Catalonia, Spain). The river water included small concentrations of N and P and dissolved organic matter mainly due to human activities (e.g. use of fertilizers). The Ter headwaters are located in the Pyrenees. Its basin has alkaline-earth bicarbonate waters. Bicarbonate represents 63% of the total anions and calcium 60% of the total cations. Other ions reach relevant concentrations. Sulphate constitutes on average 24% and chloride only 13% of the total anions (Sabater et al., 1992). Water conductivity along the catchment ranges from 250 μS/cm to 950 μS/cm (CERM database). The mesocosm was located in an open field in the Museu del Ter (Manlleu, Catalonia, Spain), close to a river channel. The river water was pumped into four 1500 L mixing tanks, each of them feeding three artificial streams (i.e. pvc pipes) and flowing into 500 L tanks from where it was re-circulated. One of the tanks was left as control (i.e. containing only river water), whereas in the other three a salt treatment was applied. The salt treatments consisted in dissolving a salt-saturated stock solution (coming from a tailing heap in the River Werra basin) into river water (total salt concentration = 0.248 g/L) at different concentrations (i.e. Mod, High and Mod-p treatments). The stock solution contained 172 g/l Chloride, 15.5 g/l Potassium and 24.9 g/l Magnesium. In the moderate treatment (Mod) the salinity was 2.27 ± 0.36 g/L, and in the high treatment (High) it was 3.78 ± 0.26 g/L. These salt concentrations are typically observed in different sections of the River Werra during different periods of the year (Bäthe and Coring, 2011a, 2011b; Coring and Bäthe, 2011). Finally, in the moderate + pulses treatment (Mod-p) the salinity concentration was 1.61 ± 0.08 g/L and it was increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after the beginning of the experiment. The pulses were created by slowly adding the salt-saturated solution to the mixing tank while continuously controlling salinity with a conductimeter. After 48 h river water was added to the mixing tanks to dissolve salts and re-establish pre-pulse conditions. Since we knew the volume of water in the tanks and the salt concentration in the river water and the mixing tanks, we could calculate which volume of river water was needed to re-establish initial (i.e. pre-pulse) salt concentrations.

We collected invertebrates and cobbles from the Ter River at Les Masies de Voltregà, which is a river section located near the artificial streams that has a good water quality and mean conductivity of 350 μS/cm (CERM database). A total of 144 river cobbles were collected and transferred to the artificial streams (placing 12 per stream). Additionally, 12 macroinvertebrate samples were collected by kick-net sampling the riverbed for 1 min using a 250 μm mesh size. Each sample was emptied at the top of each stream. Thus, initial algal communities consisted in those that were attached to the river cobbles, whereas invertebrate communities were represented by those that were attached to the cobbles and those that were collected by kick-net sampling. Prior to the experiment, river water with no salt addition flowed through all of the streams for 14 days to promote the stabilization of the initial algal and invertebrate communities.

2.2. Data collection

Water and biological samples were collected at the beginning of the experiment (i.e. before adding salt) and 9, 19, 30 and 41 days after salt was added. Water samples were collected at each occasion to analyse the major ion concentrations (K^+, Mg^{2+}, Ca^{2+}, Na^+, S^{2-}, Cl^-, HCO_3^-). The samples were analysed in the laboratory of the K + S KALI GmbH (Philippsthal, Germany). Chloride and hydrogen carbonate were analysed according to the German standard methods for the examination of water, waste water and sludge [DIN 38409-H7-2 (2005), DIN 38405-D1-2 (1985)]. Other ions were analysed by ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry [DIN EN ISO 11885 (2009)]). The concentration of major algal groups in each treatment was determined by using a bbe BenthoTorch (bbe moldanke GmbH®), which allows *in situ* quantification of different algae as chlorophyll-a, namely green algae, blue-green algae (cyanobacteria) and diatoms. The calculation is carried out internally using optimised algorithms. We performed 3 measurements at each sampling occasion on a metal structure that was submersed in the 500 L tanks at the end of the streams. This structure was free from algae at the beginning of the experiment; thus algal colonization came exclusively from the artificial streams.

Two cobbles were randomly selected for diatom and invertebrate community analysis at each sampling occasion and merged into one sample to integrate spatial heterogeneity. Since each tank fed three streams, there were three replicates for each treatment. After sampling the cobbles were returned to their initial position within the respective channel and marked to avoid re-sampling them. Diatom samples were collected by scraping 10 cm^2 of each river cobble with a toothbrush and vigorously shaking it in a plastic jar containing river water. Then, they were fixed with 70% alcohol and taken to the laboratory for taxonomic identification. Diatom treatment and identification was performed following DIN EN 13946 (2014) and DIN EN 14407 (2014), together with additional books and papers on diatom taxonomy (e.g. Krammer and Lange-Bertalot, 1986, 1988, 1991a, b). Determination was carried out using light microscopes (Zeiss Axioskop and Zeiss Jenaval, magnification 1.000×). Invertebrates were sampled by scraping the cobbles' surface and preserved in ethanol 70%. The invertebrate density was calculated based on the surface area of each cobble, which was measured by wrapping them with aluminium foil and subsequently calculating the area from the weight of the foil (Bergey and Getty, 2006). By the end of the experiment (i.e. day 41), after sample collection, all gravel was washed and filtered through a net (250 μm mesh size) to collect the remaining invertebrates. Most invertebrates were identified to family level following Tachet et al. (2000), with the exception of Chironomidae and Trichoptera, which were identified to genus level following Andersen et al. (2013) and Vieira-Lanero (2000), respectively, given that they were the dominant taxa on the sampling site.

2.3. Data analysis

All statistical analyses were performed using the statistical software R (R Core Team, 2015). Repeated measures ANOVA tests were used to assess overall differences in algae concentration, diatom richness and invertebrate density and richness between treatments using the ezANOVA function in the R package ez (Lawrence, 2015). One-way ANOVAs were used to assess differences at each sampling occasion and Tukey post-hoc tests were used to assess pairwise differences between treatments. Given that initial communities tend to be different for each channel in this kind of mesocosm studies (e.g. Cañedo Argüelles et al., 2012, 2014; Grantham et al., 2012), abundance and richness were analysed as the change from initial conditions for each channel (i.e. abundance/richness at a given time in a given channel divided by the abundance/richness at time 0 at that given channel). That allowed us to assess the effects of salt treatments on the rate of change in abundance and richness along time. Model residuals were checked for normality and homoscedasticity assumptions. In the cases where normality was not met, data were square root transformed and alpha was set to 0.01 to reduce the risk of finding false positives. In the case of heteroscedasticity, a robust statistical test was used as a post hoc test with adjustment of p-values for multiple pairwise comparisons between treatment levels (Herberich et al., 2010). The changes in diatom and invertebrate community composition were analysed by constructing a Bray-Curtis dissimilarity matrix using the function “bcdist” in the R package “ecodist” (Goslee and Urban, 2007). Then the distance of each treatment to the control treatment was plotted along time and linear models were built to look for significant trends using adjusted R^2. Finally, Indicator Species Analysis (Dufrêne and Legendre, 1997) was performed to look for taxa significantly associated to each treatment and/or group of treatments using the “multipatt” function in R package “indicspecies” (Cáceres and Legendre, 2009). This analysis chooses the combination of treatments with a highest association value to each taxa based on its abundance and frequency in each treatment. Best matching patterns are tested for statistical significance of the associations (i.e. a p-value is obtained).

Using information on diatom and invertebrate communities, we calculated the following biotic indices: IPS (Coste, 1987) for diatoms and IBMWP (Alba-Tercedor et al., 2002) and IMMi-T (Munné and Prat, 2009) for invertebrates. The IPS index is a biotic index developed on the Rhone - Mediterranean - Corsica basin and it is based on the weighted averaging equation of Zelinka and Marvan (1961) who provided integrated assessments of a range of water quality variables, including organic pollution, eutrophication, salinity and toxic materials. It has the advantage of taking into account all the species present in the inventories and it gives a rating for water quality ranging from 1 (very polluted waters) to 20 (pristine waters). Diatom taxa were also classified according to their salinity relationship using the classic halobian system (Hustedt, 1953, 1957; Ziemann, 1999). The halobic index (Ziemann, 1971, 1999) is also based on the halobian system, and it takes into account the mean of the proportion of the species groups. It is a common tool to classify the degree of salinization in Germany. The IBMWP index assigns a score to each invertebrate family according to its sensitivity to pollution, i.e. it is significantly correlated with nutrient and some ion concentrations (Alba-Tercedor et al., 2002). The index relies on a single metric: the sum of the sensitivity scores of the invertebrate families that are present in a given sample. The IMMi-T index uses a combination of the following metrics: number of families, number of Ephemeroptera + Plecoptera + Trichopetra, IASPT (i.e. IBMWP value/number of families) and log (Sel EPTCD + 1), which is based on pollution sensitive Ephemeroptera, Plecoptera, Trichoptera, Coleoptera and Diptera.

The software OMNIDIA (Lecointe et al., 1993) was used to calculate the IPS index and the software MAQBIR (Munné and Prat, 2009) was used to calculate the IBMWP and IMMi-T indices. According to the indices scores each sample was assigned to one of the five quality classes established by the Water Framework Directive (WFD) (European Commission, 2000): high, good, moderate, poor and bad. Good ecological status represents the target value that all European surface waters have to achieve to ensure ecological protection (Birk et al., 2012). Water bodies in moderate, poor or bad condition need to be managed so that good quality is restored. If good quality is not achieved, economic and legal sanctions can be applied (Birk et al., 2012). Assessment of ecological status is based on measuring deviation from a reference (undisturbed) conditions. The boundaries between quality classes are dependent on the typology of the ecosystem, since each type has different reference communities.

Table 1
Concentration (g/L) of major ions in water samples collected from the different treatments and at different times (e.g. Day 0 = first day of sampling, before treatment started; Day 9 = 9 days after treatment started). Con = control treatment (i.e. river water; salinity = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). * = water sample collected during salt pulse in treatment Mod p.

	K^+	Mg^{2+}	Ca^{2+}	Na^+	S^{2-}	Cl^-	HCO_3^-	Total
Day 0								
Con	0.004	0.008	0.050	0.014	0.015	0.015	0.142	0.248
Mod	0.003	0.008	0.047	0.012	0.015	0.013	0.140	0.238
Mod p	0.003	0.008	0.044	0.013	0.016	0.013	0.130	0.227
High	0.002	0.008	0.047	0.011	0.016	0.013	0.136	0.233
Day 9								
Con	0.003	0.007	0.041	0.010	0.012	0.011	0.121	0.205
Mod	0.081	0.138	0.047	0.433	0.105	0.932	0.134	1.870
Mod p	0.061	0.106	0.057	0.330	0.085	0.702	0.154	1.495
High	0.148	0.245	0.052	0.781	0.179	1.674	0.139	3.218
Day 19*								
Con	0.003	0.007	0.043	0.012	0.013	0.014	0.126	0.218
Mod	0.090	0.153	0.065	0.481	0.116	1.039	0.186	2.130
Mod p	0.097	0.166	0.060	0.523	0.127	1.126	0.168	2.267
High	0.160	0.268	0.062	0.851	0.196	1.817	0.159	3.513
Day 26*								
Mod p	0.095	0.161	0.057	0.503	0.123	1.082	0.148	2.169
Day 30*								
Con	0.003	0.008	0.047	0.014	0.015	0.018	0.139	0.244
Mod	0.101	0.174	0.062	0.549	0.133	1.182	0.171	2.372
Mod p	0.095	0.164	0.059	0.516	0.125	1.115	0.173	2.247
High	0.183	0.304	0.066	0.952	0.219	2.046	0.170	3.940
Day 41								
Con	0.003	0.009	0.045	0.014	0.016	0.017	0.123	0.227
Mod	0.117	0.198	0.071	0.622	0.151	1.346	0.191	2.696
Mod p	0.071	0.124	0.057	0.384	0.097	0.827	0.159	1.719
High	0.203	0.342	0.076	1.064	0.249	2.310	0.189	4.433

In our case, reference conditions were selected according to the river typology from where the algal and invertebrate communities were sampled.

3. Results

3.1. Ion concentrations

Ion concentrations were similar among channels before treatment started and increased to 2.27 ± 0.36 g/L and 3.78 ± 0.26 g/L in the Moderate and High treatments, respectively. In the Moderate-p total ion concentration was 1.61 ± 0.08 g/L and it increased to 2.23 ± 0.03 g/L (i.e. similar to Moderate treatment) during the salt pulses. The ionic proportions in the control were $HCO_3^- > Ca^{2+} > S^{2-} = Cl^- > Na^+ > Mg^{2+} > K^+$, whereas the ionic proportions in the treatments after salt addition were $Cl^- > Na^+ > Mg^{2+} > S^{2-} > K^+ > HCO_3^- > Ca^{2+}$. The concentrations of ions increased in all treatments during time except for the control (Table 1).

3.2. Algal communities

Significant overall differences in the concentration of cyanobacteria between treatments were found at all sampling dates: day 0 ($F = 11.36$; p-value = 0.003), day 9 ($F = 15.5$; p-value = 0.001), day 19 ($F = 8.44$; p-value = 0.007), day 30 ($F = 27.13$; p-value = 1.15×10^{-4}) and day 41 ($F = 11.35$; p-value = 0.003). Post-hoc tests revealed significantly higher concentrations in the Mod and Mod-p treatments than in control and High at the beginning of the study, before the salt was added (Fig. 1). Once the salt was added, cyanobacteria concentrations were always higher in the salt treatments than in the control, except for the Mod-p treatment, in which cyanobacteria concentrations went down to control level once the salt pulses started (Fig. 1). Significant overall differences in the concen-

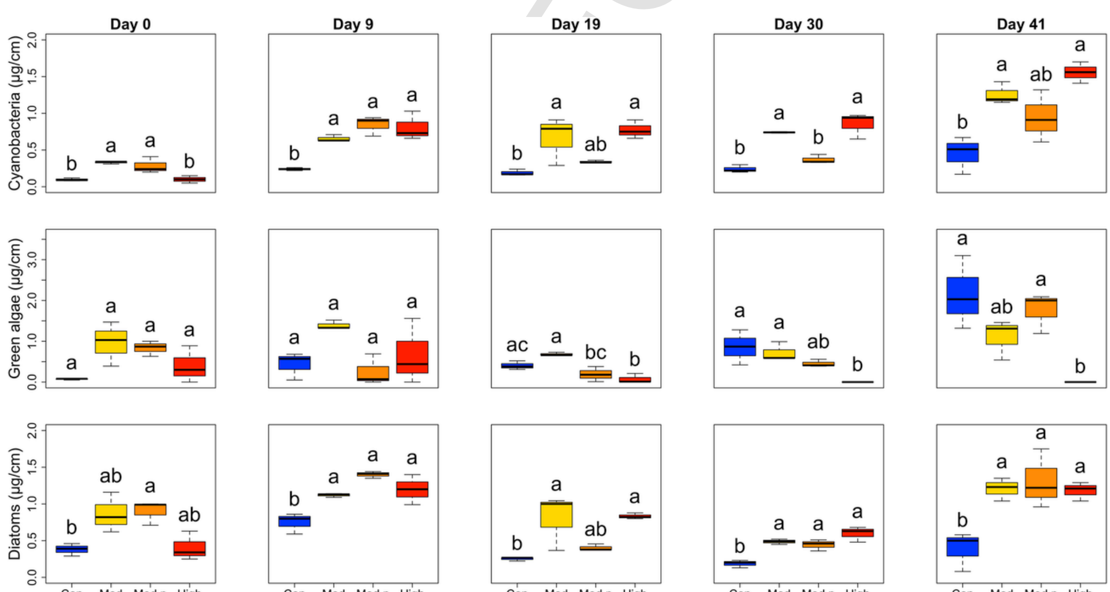

Fig. 1. Concentration of cyanobacteria, green algae and diatoms in the experimental channels along time expressed in µg per square centimetre. Each graph represents a different sampling date and each boxplot a different treatment. Con = control treatment (i.e. river water; salinity = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). Day 0 = before treatment started. Day 9 = 9 days after treatment started. Day 19 = 19 days after treatment started. Day 30 = 30 days after treatment started. 41 = 41 days after treatment started. In the Mod-p treatment salinity were increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after treatment started. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

tration of green algae between treatments were found only 19 ($F = 13.83$; p-value = 0.002), 30 ($F = 6.89$; p-value = 0.013) and 41 ($F = 8.21$; p-value = 0.008) days after treatment started. According to post-hoc tests the concentration of green algae was significantly lower in the High treatment than in the rest of the treatments at days 19, 30 and 41, except for the Mod – p at days 19 and 30 and the Mod treatment at day 41 (Fig. 1). Significant overall differences in the concentration of diatoms between treatments were found at all sampling dates: day 0 ($F = 6.50$; p-value = 0.0154), day 9 ($F = 13.62$; p-value = 0.002), day 19 ($F = 8.10$; p-value = 0.008), day 30 ($F = 17.6$; p-value = 6.97×10^{-4}) and day 41 ($F = 7.93$; p-value = 0.009). According to post-hoc tests the concentration of diatoms was significantly lower in the control than in the treatments at days 9, 30 and 41 (Fig. 1), whereas at days 0 and 19 the differences between treatments were more variable (Fig. 1).

We recorded a total of 185 diatom taxa. According to repeated measures ANOVA diatoms richness did not changed significantly along time ($F = 0.33$, p-value = 0.852) and it was not affected by the salt treatments ($F = 0.25$, p-value = 0.856) (Fig. 2). However, diatom community composition was significantly affected by the salt treatments (Fig. 3). Concordantly, the Indicator Species Analysis (IndVal) revealed that 22 diatom species were significantly associated with different treatment combinations (Table 2). Five species were significantly associated with the control group, indicating that they were sensitive to salt pollution: *Cymbella excisa* var *excisa*, *Gomphonema minutum*, *Gomphonema undet*, *Gomphonema pumilum* and *Planothidium lanceolatum*. On the contrary different *Navicula* and *Nitzschia* species seemed to be salt tolerant, since they were significantly associated with the salt treatments (Table 2). According to the IPS index (Table 3) the control was in a good (days 0, 9, and 19) to moderate (days 30 and 41) status, whereas the rest of the channels were mostly in a moderate status except for the Mod-p (good at day 0) and the High (poor at day 41) treatments. In spite of a given variability within the samples, we found clear differences in the proportion of the halobian groups between the treatments. The share of halophilous and mesohalobic diatoms (mean value of all samples per treatment) increased continuously from 8.3% (control) to 18.2% in the High treatment (12.8% Mod, 15.1% Mod P) attended by a decline in oligohalobic and indifferent diatoms. Concordantly the mean value of the halobic index raised from 9.5 in the control to 12.7 in the Mod, 15.4 in the Mod –p and 17.5 in the High treatment indicating "typical freshwater" for the control and "freshwater with increased salt content" for all salt treatments.

3.3. Invertebrate communities

We recorded a total of 26 taxa, the most abundant being Chironomidae (mean relative abundance = 38.82 ± 0.55%), Trichoptera (mean relative abundance = 30.75 ± 0.29%) and Simuliidae (mean relative abundance = 15.46 ± 0.13%). According to repeated measures ANOVA invertebrate density was not affected by treatment ($F = 0.32$, p-value = 0.812), but it changed significantly with time

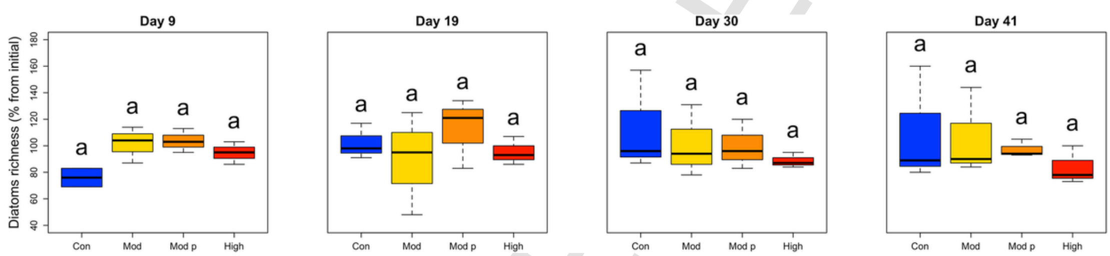

Fig. 2. Diatom richness in the experimental channels along time expressed as the richness at a given day divided by the initial richness. This was calculated for each channel separately to account for variability in initial communities between channels and to analyse differences in variation from initial conditions for each treatment and replicate. Each graph represents a different sampling date and each boxplot a different treatment. Con = control treatment (i.e. river water; salinity = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). Day 0 = before treatment started. Day 9 = 9 days after treatment started. Day 19 = 19 days after treatment started. Day 30 = 30 days after treatment started. 41 = 41 days after treatment started. In the Mod-p treatment salinity were increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after treatment started.

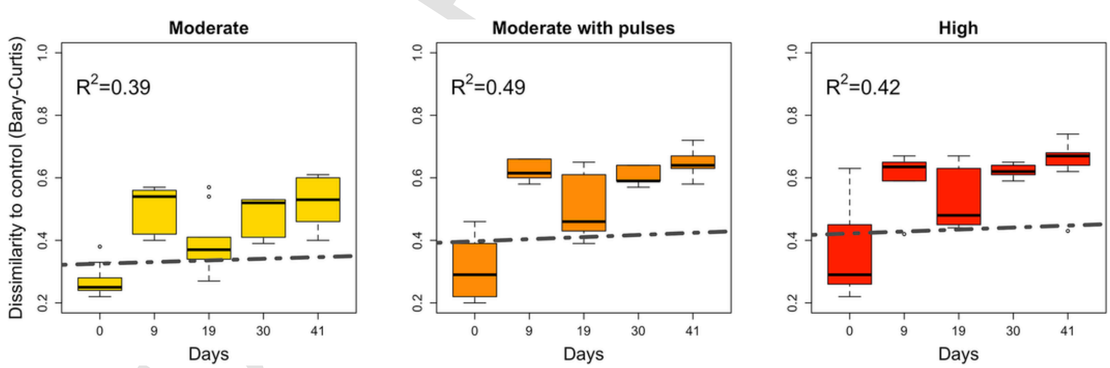

Fig. 3. Changes in diatom community composition in the experimental channels along time, expressed as the dissimilarity between treatment and control communities. This was calculated for each channel separately to account for variability in initial communities between channels and to analyse differences in variation from initial conditions for each treatment and replicate. Each graph represents a different sampling date and each boxplot a different treatment. Con = control treatment (i.e. river water; salinity = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). Day 0 = before treatment started. Day 9 = 9 days after treatment started. Day 19 = 19 days after treatment started. Day 30 = 30 days after treatment started. 41 = 41 days after treatment started. In the Mod-p treatment salinity were increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after treatment started.

Table 2
Results from the Indicator Species Analysis, showing the taxa significantly associated to each IV = indicator value according to the abundance and frequency of each taxa in each treatment. *p*-value = statistical significance of the associations. Con = control treatment (i.e. river water; salinity = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). Day 0 = before treatment started. Day 9 = 9 days after treatment started. Day 19 = 19 days after treatment started. Day 30 = 30 days after treatment started. 41 = 41 days after treatment started. In the Mod-p treatment salinity were increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after treatment started.

	Group	IV	p-value
Diatoms			
Cymbella excisa var *excisa*	Con	0.519	0.005
Gomphonema minutum	Con	0.825	0.005
Gomphonema undet	Con	0.645	0.020
Gomphonema pumilum	Con	0.431	0.045
Planothidium lanceolatum	Con	0.545	0.015
Diatoma moniliformis	Mod	0.457	0.025
Fragilaria capucina group	Con + Mod	0.896	0.005
Fragilaria nanana	Mod + Mod p	0.553	0.020
Stephanodiscus hantzschii	Mod + High	0.72	0.025
Hippodonta capitata	Mod p + High	0.698	0.005
Simonsenia delognei	Mod p + High	0.703	0.005
Cymbella microcephala	Con + Mod + Mod p	0.603	0.020
Fragilaria ulna var *ulna*	Con + Mod + Mod p	0.877	0.005
Nitzschia sociabilis	Con + Mod + Mod p	0.658	0.005
Fragilaria familiaris	Con + Mod + High	0.82	0.005
Nitzschia clausii	Con + Mod p + High	0.783	0.005
Navicula undet	Mod + Mod p + High	0.811	0.015
Naviculadicta absoluta	Mod + Mod p + High	0.752	0.020
Navicula veneta	Mod + Mod p + High	0.951	0.005
Nitzschia gracilis	Mod + Mod p + High	0.767	0.030
Nitzschia microcephala	Mod + Mod p + High	0.880	0.015
Sellaphora pupula var *pupula*	Mod + Mod p + High	0.885	0.005
Invertebrates			
Eukiefferella minor fitkau	Mod p	0.95	0.005
Baetis gr rhodani	Con + Mod + Mod p	0.825	0.030

($F = 139$, p-value $= 9.27 \times 10^{-20}$) and there was a significant time × treatment interaction ($F = 3.59$, p-value $= 1.88 \times 10^{-3}$). Changes in invertebrate density were only significant between treatments at day 19 ($F = 9.50$; p-value = 0.005), when the reduction in initial density was significantly lower in the Mod-p treatment than in the rest of the treatments (Fig. 4). Post-hoc tests also revealed a significantly higher reduction in invertebrate density in the High treatment at day 30 then in the rest of the treatments (Fig. 4). According to repeated measures ANOVA invertebrate richness was not affected by treatment ($F = 0.32$, p-value = 0.812), but it changed significantly with time ($F = 28.37$, p-value $= 4.03 \times 10^{-20}$) and there was significant time × treatment interaction ($F = 2.62$, p-value $= 1.46 \times 10^{-2}$). Similarly, one-way ANOVA and Tukey tests revealed no significant differences in invertebrate richness between treatments throughout the experiment (Fig. 4). However, invertebrate community composition differed significantly between the control and the salt treatments (Fig. 5). According to IndVal analysis only two invertebrate species were significantly associated to a treatment or to treatments combination (Table 2): *Eukiefferella minor fitkau* and *Baetis gr. rhodani*. The former was significantly more abundant and frequent in the Mod p treatment, whereas the latter was significantly less abundant and frequent in the High treatment. According to the IBMWP index (Table 3) all the salt treatments had a poor status, except for Mod at day 0 (moderate status) and the High at day 30 (bad status). According to the IMMi-T index (Table 3), all treatments had a good status at day 0 and a moderate status at days 9 and 19. At day 30 the control and the Mod p treatment had a good status, the Mod treatment had a moderate status and the High treatment had a poor status.

Table 3
Results from the calculation of different biotic indices using the diatom and invertebrate samples collected from the cobbles in the experimental channels. IPS = Specific Pollution Sensitivity Index, based on diatom data. IBMWP = based on invertebrate presence/absence data. IMMi-T = based on invertebrate density (individuals per m^2) data. According to the indices scores the channels were assigned to one of the five quality classes established by the Water Framework Directive: high (blue), good (green), moderate (yellow), poor (orange) and bad (red). As reference conditions, we selected the river typology from where the algal and invertebrate communities were sampled and transferred to the artificial streams. Con = control treatment (i.e. river water; salinity = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). Day 0 = before treatment started. Day 9 = 9 days after treatment started. Day 19 = 19 days after treatment started. Day 30 = 30 days after treatment started. 41 = 41 days after treatment started. In the Mod-p treatment salinity were increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after treatment started.

	Diatoms	Invertebrates	
	IPS	IBMWP	IMMi-T
Day 0			
Con	0.72	0.17	0.71
Mod	0.71	0.30	0.81
Mod p	0.55	0.27	0.71
High	0.60	0.27	0.76
Day 9			
Con	0.88	0.20	0.65
Mod	0.59	0.23	0.64
Mod p	0.69	0.28	0.70
High	0.48	0.22	0.69
Day 19			
Con	0.73	0.13	0.60
Mod	0.55	0.20	0.61
Mod p	0.60	0.13	0.53
High	0.63	0.15	0.69
Day 30			
Con	0.62	0.22	0.71
Mod	0.72	0.17	0.67
Mod p	0.50	0.24	0.74
High	0.58	0.03	0.40
Day 41			
Con	0.59		
Mod	0.77		
Mod p	0.67		
High	0.44		

Total invertebrate abundance in the cobbles and the channels at the end of the experiment was: 63, 105, 152 and 43 individuals for the control and the Mod p, Mod and High treatments, respectively. Total invertebrate richness was 12, 15, 13 and 5, respectively. The Trichoptera *Hydropsyche exocellata* was the most abundant taxa (relative abundances: Control = 44%; Mod = 42% and High = 88%), except for the Mod p treatment, which was dominated by *Eukiefferella minor fitkau* (relative abundance = 55%).

4. Discussion

Although previous studies had already tested the effects of increased salinity on algal and invertebrate communities, this is the first time that a real potash mining effluent is tested. As hypothesized, the High treatment (mean salinity = 3.78 ± 0.26 g/L) had the strongest effect on the algal and invertebrate communities. However the Mod (salinity = 2.27 ± 0.36 g/L) and Mod-p (salinity during normal condi-

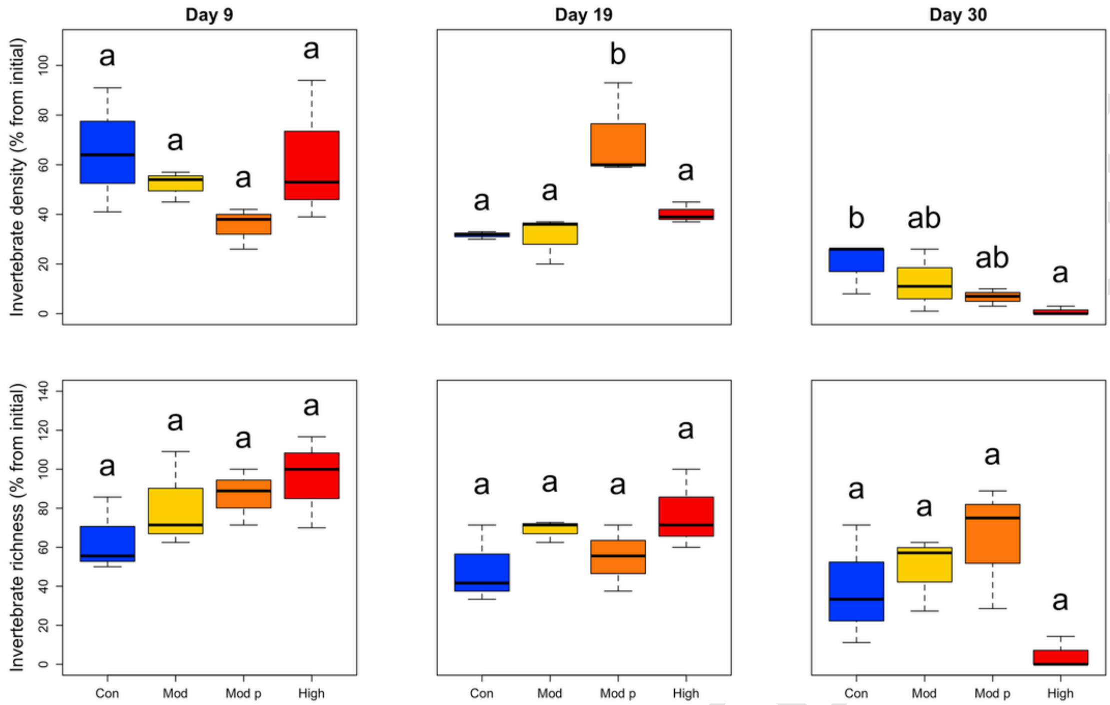

Fig. 4. Invertebrate density (upper graphs) and richness (lower graphs) in the experimental channels along time expressed as the density/richness at a given day divided by the initial density/richness. This was calculated for each channel separately to account for variability in initial communities between channels and to analyse differences in variation from initial conditions for each treatment and replicate. Each graph represents a different sampling date and each boxplot a different treatment Con = control treatment (i.e. river water. (salinity) = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). Day 0 = before treatment started. Day 9 = 9 days after treatment started. Day 19 = 19 days after treatment started. Day 30 = 30 days after treatment started. 41 = 41 days after treatment started. In the Mod-p treatment salinity were increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after treatment started.

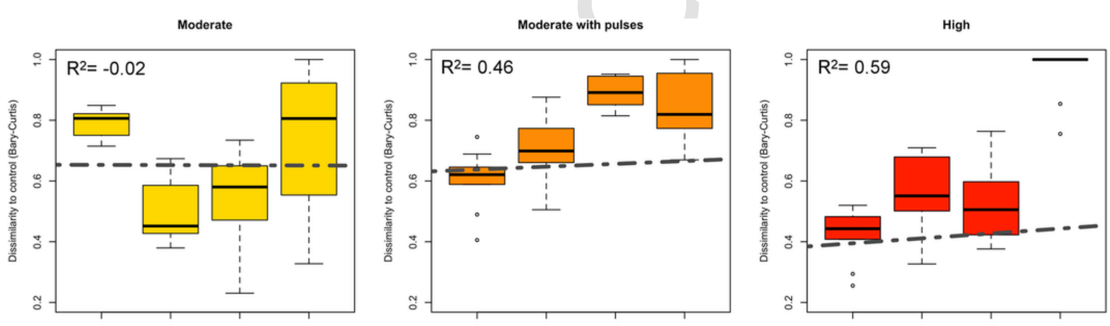

Fig. 5. Changes in aquatic invertebrate community composition in the experimental channels along time, expressed as the dissimilarity between treatment and control communities. This was calculated for each channel separately to account for variability in initial communities between channels and to analyse differences in variation from initial conditions for each treatment and replicate. Each graph represents a different sampling date and each boxplot a different treatment Con = control treatment (i.e. river water; salinity = 0.23 ± 0.02 g/L); Mod = moderate treatment (salinity = 2.27 ± 0.36 g/L); Mod-p = low treatment with pulses up to moderate concentration (salinity during normal conditions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L); High = high treatment (salinity = 3.78 ± 0.26 g/L). Day 0 = before treatment started. Day 9 = 9 days after treatment started. Day 19 = 19 days after treatment started. Day 30 = 30 days after treatment started. 41 = 41 days after treatment started. In the Mod-p treatment salinity were increased to 2.23 ± 0.03 g/L during 3 pulses of 48 h of duration applied 19, 28 and 35 days after treatment started.

tions = 1.61 ± 0.08 g/L; salinity during salt pulses = 2.26 ± 0.03 g/L) treatments also had significant effects on some parameters (e.g. cyanobacteria and diatom concentrations). Overall, the effects of salt addition were time-dependent, being more clear by the end of the study. This is something that had been previously reported in other mesocosm studies (Cañedo Argüelles et al., 2014; Cañedo Argüelles et al., 2012), and that is most likely related with the ability of freshwater organisms to resist high salinities for a certain period of time (e.g. by osmoregulation). However, it could also be related with the increase in ion concentrations in the channels along time, which was probably caused by water evaporation. Taxa richness was unaffected by treatment. This is also in agreement with previous mesocosm studies (Cañedo Argüelles et al., 2014; Cañedo Argüelles et al., 2012) and it could be caused by intraspecific variations in salt sensitivity (i.e. some resistant individuals not being killed by treatment). For example, Kefford et al. (2007) showed that salinity tolerance varied across life stages of the same species, and Sala et al. (2016) found that populations of *Hydropsyche exocellata* historically exposed to higher salinities had a greater tolerance to salt pollution. Finally, the short (i.e. 48 h long) salt pulses had a weak effect on the algal and invertebrate communities. It is important to notice that the concentration of cyanobacteria and green algae in the Mod-p treatments was not different from the control. Also, the reduction in invertebrate density in this treatment was lower than in rest of the treatments at day 19, and lower than in the high treatment at day 30. Thus, our results suggest that the lower salt concentration in this treatment (salinity = 1.61 ± 0.08 g/L) was more important than the salt pulses (rising salinity to levels comparable to the Mod treatment for 48 h) in determining changes in the algal and invertebrate communities. Concordantly, Cañedo Argüelles et al. (2014) found that very short salt pulses (3 h of duration) had a relatively weak effect on algal and invertebrate communities. As suggested in that study, the lack of significant effect of the salt pulses could be related with the time between pulses (7–9 days), which should have been long enough to allow organisms to recover. For example, algae and invertebrates have been shown to recover from pesticides just a few days after exposure (Heckmann and Friberg, 2005; Proia et al., 2011).

4.1. Algae

The effect of the salt treatments on algal biomass seemed to be time-dependent, with clear differences between treatments by the end of the study (i.e. 41 days after the salt treatment started). We found higher algal biomass (expressed as chlorophyll *a* concentration per cm^2) in salt treatments than in control. This result is consistent with findings in the river Werra, Germany (Coring and Bäthe, 2011) where field measurements using a comparable technique supported the hypothesis of fertilizing effects (i.e. promoting algal growth) of saline wastewater and high potassium concentrations. The salt treatments caused a significant dominance of cyanobacteria and diatoms, whereas green algae were significantly reduced in the High treatment. In the salinized river Werra the abundance of diatoms, cyanobacteria and green algae changed from year to year and there was no significant difference in the ratio of the different algae groups between salinized and unpolluted segments of the river (Coring and Bäthe, 2011). Thus, additional investigations will be necessary to demonstrate potential connections between salinity and the quantitative development of different algae groups. For example, in combination with other variables, the development cycle of the phytobenthos may have an additional or even stronger effect on the composition of the benthic algal biomass than a single stressor like salinity. The treatments had significant effects on the diatom community composition, with *Cymbella excisa* var *excisa*, *Planothidium lanceolatum* and some *Gomphonema* species being salt sensitive and different *Navicula* and *Nitzschia* species being salt tolerant. This is in agreement with the ecology of the species, since the first group corresponds to taxa generally inhabiting clear waters, whereas the *Navicula* and *Nitzschia* comprise pollution-tolerant species that can persist in eutrophic and saline waters (Hofmann et al., 2013). Several *Navicula* and *Nitzschia* were also dominant in heavily salt polluted rivers in Germany (Hofmann, 1997; Coring and Bäthe, 2011) and benefited from oligohalobic and mesohalobic conditions. The documented changes in the proportion of the halobian groups and the halobic index fit to previous field studies. For example, Ziemann et al. (2001) showed that the abundance of oligohalophilic and increasing mesohalophilic and polyhalophilic diatoms was highly dependent on river salinization. Schulz (2016) found that increased salt concentrations led to a replacement of haloxenic taxa by halophilic, meso- and polyhalobic forms. Additionally, Pudwill and Timm, 1997 determined a threshold concentration for the effects of salt on diatoms between about 100 and 200 mg l^{-1} chloride or sulphate. They showed that salinization is indicated by diatoms before any other effects on the biota (e.g. macroinvertebrates) can be observed. Thus, our findings conform to field observations, suggesting that salinity can be a key driver of diatom community composition. The response of the diatom community to the salt treatment contrasts with a previous study using 3 h salt pulses over a 16 days period (Cañedo Argüelles et al., 2014), in which no significant effects of treatment on the diatom community were found. This could be related to the longer duration of the present experiment and the fact that we recorded a higher taxa richness (185 taxa vs. 102 taxa recorded in Cañedo Argüelles et al., 2014), including taxa sensitive to pollution. In general, there is still a strong need for further investigations on the reactions of diatoms to salinization. Even if diatoms are able and useful to determine early stages of salinization in aquatic ecosystems, a common understanding of cause and effect relationships is still under discussion. Also, it is not clear whether and to what extent changes in the species community structure can affect the functionality of the ecosystem (Cañedo-Argüelles et al., 2013).

4.2. Invertebrates

The salt treatments had an effect on invertebrate community composition that was mainly related with changes in the abundance of certain taxa. For example, the Ephemeroptera *Baetis gr. rhodani* was significantly less abundant and frequent in the High treatment than in the rest of treatments. This is most likely related to the salt sensitivity of this species, which was significantly more frequent and abundant in the control than in the salt treatments in a previous similar study using stream mesocosms (Cañedo Argüelles et al., 2012). Moreover, Ephemeropterans have been reported to be salt sensitive by several field studies (e.g. Clements and Kotalik, 2016; Hartman et al., 2005; Johnson et al., 2015; Kefford et al., 2011; Kennedy et al., 2003; Pond, 2010). The Chironomidae *Eukiefferella minor fitkau* was more abundant and frequent in the Mod-p than in the rest of the treatments. According to previous studies, the genus *Eukiefferella* includes salt tolerant species. For example, it has been recorded in saline lakes (Cannings and Scudder, 1978), saline ponds (Dickson et al., 2014) and the mouth of estuaries (Williams and Hamm, 2002) in Canada. In a recent study using artificial streams to assess the effect of salt pulses on aquatic ecosystems near our study area (Cañedo Argüelles et al., 2014), *Eukiefferella* had an euryhaline behaviour, being present in all treatments and showing no clear salinity preferences. Thus, the dominance of *Eukiefferella minor fitkau* in the Mod-p treatment could be attributed to a greater abundance of this species in the cobbles that were transferred from the river to the Mod-p experimental channels, since Chironomidae can follow random patchy distributions in streams (Schmid, 1993; Tokeshi and Townsend, 1987). Overall invertebrate richness was not affected by salinization, contrasting with field studies that have reported decreasing richness along salinity gradients (e.g. Braukmann and Böhme, 2011; Kefford et al., 2011; Schulz, 2016). As suggested by previous mesocosm experiments (Cañedo Argüelles et al., 2012, 2014) this could be related with the mesocosm communities being a sub-set of natural communities and thus not containing some salt-sensitive species (for example we only recorded 2 different Ephemeroptera taxa and no Plecoptera). Also, the cited field studies explored long-term exposure to salinization. Therefore, species filtering can be expected to be stronger than in short term salinity exposure experiments (Kefford et al., 2016).

4.3. Indices

The biotic indices showed a weak response to treatment, with only the High treatment causing a consistent (i.e. according to all indices) reduction in the ecological quality of the streams and only by the end of the study. Accordingly, Cañedo Argüelles et al. (2012) only found a reduction in the ecological status of the artificial streams subjected to high salt concentrations (i.e. 5 mS $cm^{-1} \approx 3$ g L^{-1}) at the end of their study (i.e. 72 h of exposure). The weak response showed by biological indices could be related to the negligible effect that salt treatments had on taxa richness, since this is one of the key metrics that most biological indices rely upon. Thus, we suggest that specific indices for detecting salt pollution need to be developed and implemented in monitoring programmes. An example can be found in Germany, where the DWA (German Association for Water, Wastewater and Waste) Working Group "Salt loading of flowing waters" recently developed a salinity index based on a precise auto-ecological characterisation of macroinvertebrates over a wide chloride gradient (Coring et al., 2016). The index enables the differentiation and classification of anthropogenic salinization into a five-class system. These classes are limited by threshold values, where significant changes in benthic macroinvertebrate communities can be observed. An intersection of the salinity index with the ecological status classes in terms of the European Water Framework Directive (WFD) has still not taken place but may be of great interest for the management of salinized rivers and streams. This should be done at least in rivers and streams impacted by mining, but given that freshwater salinization is a widespread phenomenon caused by a wide variety of human activities (Cañedo-Argüelles et al., 2013; Cañedo-Argüelles et al., 2016), its development and implementation in all rivers and streams is recommended.

5. Conclusions

Overall, our results suggest that potash mining has the potential to significantly alter biological communities of surrounding rivers and streams. We found that salt concentrations as low as 1.61 ± 0.08 g/L can rapidly increase cyanobacteria concentration, promote the dominance of halophilous and mesohalobic diatoms and alter the invertebrate community composition. Although only the highest salt concentration (3.78 ± 0.26 g/L) led to a significant decline of invertebrate abundance and a degradation of the ecological status, it should be noticed that the biological communities that were present in the artificial streams in this study were a subset of the communities that can be found in the river: they were poorer in species and dominated by tolerant species like *Hydropsyche exocellata* (Bonada et al., 2004; Sala et al., 2016). Therefore the impact of the salt treatments on natural communities can be expected to be stronger than the one reported here and a salt concentration limit of 1.61 ± 0.08 g/L can be considered as conservative. Previous studies (Cañedo Argüelles et al., 2012; Dunlop et al., 2008; Kefford et al., 2011; Pinder et al., 2005) suggest that a salinity of around 3 g L^{-1} could be the threshold for detecting strong effects on aquatic invertebrate communities (i.e. drastic reductions in abundance and/or number of surviving taxa). However, as observed in this study and previous investigations (e.g. Böhme, 2011; Cormier et al., 2013; Marshall and Bailey, 2004), lower salinities can have significant effects on community composition. The effect of non-acute-toxic salt pulses seemed to be negligible, since there were minor differences between the effect of the Mod and the Mod-p treatments on the algal and invertebrate communities, and there was small variation in the effects of the Mod-p treatment before and after the pulses started. Although this study and a previous investigation in artificial streams (Cañedo Argüelles et al., 2014) suggest that short pulses (i.e. few hours of duration) of relatively high salt concentrations should have weak effects on freshwater organisms, the results of Marshall and Bailey (2004) should also be taken into account. They conducted field experiments to examine the effects on macroinvertebrates of increased salt concentration (around 1 and 2 g L^{-1}) and mode of salt water release (continuous press release of approximately 1.5 g L^{-1} and four, separate pulses of approximately 3.4 g L^{-1}), and found that delivering short pulses of high salt concentration was more harmful to aquatic invertebrates than delivering the same salt load at a low concentration over a longer period of time. Unfortunately, since we did not use this approach in our study (i.e. delivering the same salt load under different salt water release schemes) our results are not comparable to those of Marshall and Bailey (2004). Thus, this is an issue that requires further study (e.g. exploring pulses of different duration and magnitude) before strong management recommendations can be provided. In this regard, mesocosm experiments can be a powerful tool. Future studies should try to better incorporate natural habitat heterogeneity and the biodiversity found in natural communities. Also, testing the response of different initial communities (e.g. headwater streams versus lowland rivers) would help us to better understand the potential effects of potash mining (and salt pollution in general) on river ecosystems.

Acknowledgements

The research leading to these results has received funding from the People Program (Marie Curie Actions) of the Seventh Framework Program of the European Union (FP7/2007–2013) under grant agreement no. 600388 of REA (TECNIOspring Program) and the Agency for Competitiveness and Business of the Government of Catalonia, ACCIÓ. Part of the diatom samples were prepared and identified at the Algological Laboratory of the Botanical Unit (University of Barcelona) led by Dr. Jaume Cambra, authors are very grateful for his collaboration. We want to thank Miquel Sala for his help in the field and the staff at Museu del Ter (especially Agustí Bofill and David Rebollo) and at the CERM (Center for the Study of Mediterranean Rivers) for their assistance during the experiment.

References

Alba-Tercedor, J., Jáimez-Cuéllar, P., Álvarez, M., Avilés, J., Bonada, N., Casas, J., Mellado, A., Ortega, M., Pardo, I., Prat, N., Rieradevall, M., Robles, S., Sáinz-Cantero, C., Sánchez-Ortega, A., Suárez, M.L., Vidal-Abarca, M.R., Vivas, S., Zamora-Muñoz, C., 2002. Caracterización del estado ecológico de ríos mediterráneos ibéricos mediante el índice IBMWP (antes BMWP'). Limnetica 21.

Andersen, T., Cranston, P.S., Epler, J.H., 2013. Chironomidae of the Holarctic Region: Keys and Diagnoses. Part 1-Larvae. Insect Systematics and Evolution Supplements.

Bäthe, J., Coring, E., 2011a. Biological effects of anthropogenic salt-load on the aquatic Fauna: a synthesis of 17 years of biological survey on the rivers Werra and Weser. Limnol. - Ecol. Manag. Inl. Waters 41, 125–133. doi:http://dx.doi.org/10.1016/j.limno.2010.07.005.

Bäthe, J., Coring, E., 2011b. Biological effects of anthropogenic salt-load on the aquatic fauna: a synthesis of 17 years of biological survey on the rivers Werra and Weser. Limnologica 41, 125–133.

Bergey, E., Getty, G., 2006. A review of methods for measuring the surface area of stream substrates. Hydrobiologia 556, 7–16. http://dx.doi.org/10.1007/s10750-005-1042-3.

Bian, Z., Miao, X., Lei, S., Chen, S., Wang, W., Struthers, S., 2012. The challenges of reusing mining and mineral-processing wastes. Science (80-.) 337, 702–703.

Birk, S., Bonne, W., Borja, A., Brucet, S., Courrat, A., Poikane, S., Solimini, A., van de Bund, W., Zampoukas, N., Hering, D., 2012. Three hundred ways to assess Europe's surface waters: an almost complete overview of biological methods to implement the Water Framework Directive. Ecol. Indic. 18, 31–41.

Böhme, D., 2011. Evaluation of brine discharge to rivers and streams: methodology of rapid impact assessment. Limnologica 41, 80–89.

Bonada, N., Zamora-Munoz, C., Rieradevall, M., Prat, N., 2004. Ecological profiles of caddisfly larvae in mediterranean streams: implications for bioassessment methods. Environ. Pollut. 132, 509–521.

Braukmann, U., Böhme, D., 2011. Salt pollution of the middle and lower sections of the river Werra (Germany) and its impact on benthic macroinvertebrates. Limnol. - Ecol. Manag. Inl. Waters 41, 113–124. doi:http://dx.doi.org/10.1016/j.limno.2010.09.003.

Cáceres, M. De, Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574. http://dx.doi.org/10.1890/08-1823.1.

Cañedo Argüelles, M., Bundschuh, M., Gutiérrez-Cánovas, C., Kefford, B.J., Prat, N., Trobajo, R., Schäfer, R.B., 2014. Effects of repeated salt pulses on ecosystem structure and functions in a stream mesocosm. Sci. Total Environ. 476–477, 634–642.
Cañedo Argüelles, M., Grantham, T.E., Perrée, I., Rieradevall, M., Céspedes-Sánchez, R., Prat, N., 2012. Response of stream invertebrates to short-term salinization: a mesocosm approach. Environ. Pollut. 166, 144–151.
Cañedo-Argüelles, M., Hawkins, C.P., Kefford, B.J., Schäfer, R.B., Dyack, B.J., Brucet, S., Buchwalter, D., Dunlop, J., Frör, O., Lazorchak, J., Coring, E., Fernandez, H.R., Goodfellow, W., Achem, A.L.G., Hatfield-Dodds, S., Karimov, B.K., Mensah, P., Olson, J.R., Piscart, C., Prat, N., Ponsá, S., Schulz, C.-J., Timpano, A.J., 2016. Saving freshwater from salts. Ion-specific standards are needed to protect biodiversity. Science (80-.) 351, 8–10.
Cañedo-Argüelles, M., Kefford, B.J., Piscart, C., Prat, N., Schäfer, R.B., Schulz, C.J., **173,** 2013, 157–167. Salinisation of rivers: an urgent ecological issue. Environ. Pollut.
Cañedo-Argüelles, M., Sala, M., Peixoto, G., Prat, N., Faria, M., Soares, A.M.V.M., Barata, C., Kefford, B., 2015, 3-10. Can salinity trigger cascade effects on streams? A mesocosm approach. Sci. Total Environ. http://dx.doi.org/10.1016/j.scitotenv.2015.03.039.
Cannings, R.A., Scudder, G.G.E., 1978. The littoral Chironomidae (Diptera) of saline lakes in central British Columbia. Can. J. Zool. 56, 1144–1155.
Centre de Estudis de Rius Mediterranis (CERM) [WWW Document], URL http://www.museudelter.cat/cerm/recerca (accessed 9.7.16).
Clements, W.H., Carlisle, D.M., Lazorchak, J.M., Johnson, P.C., 2000. Heavy metals structure benthic communities in Colorado mountain streams. Ecol. Appl. 10, 626–638.
Clements, W.H., Kotalik, C., 2016. Effects of major ions on natural benthic communities: an experimental assessment of the US Environmental Protection Agency aquatic life benchmark for conductivity. Freshw. Sci. 35, 126–138. http://dx.doi.org/10.1086/685085.
Coring, E., Bäthe, J., 2011. Effects of reduced salt concentrations on plant communities in the River Werra (Germany). Limnologica 41, 134–142.
Coring, E., Bäthe, J., Dietrich, N., 2016. Indication of the salinity of flowing waters on the basis of the Macrozoobenthos. Korrenspondenz Wasserwirtsch. 2016 (9), 102–106. No. 2.
Cormier, S.M., Suter, G.W., Zheng, L., Pond, G.J., 2013. Assessing causation of the extirpation of stream macroinvertebrates by a mixture of ions. Environ. Toxicol. Chem. 32, 277–287. http://dx.doi.org/10.1002/etc.2059.
Coste, M., 1987. Etude des methods biologique quantitatives d'appreciation de la qualite des eaux. In: Rapport, Q.E., Lyon, A.F. (Eds.), Bassin Rhone-mediterannee-corse. p. 218.
Dickson, T.R., Bos, D.G., Pellatt, M.G., Walker, I.R., 2014. A midge-salinity transfer function for inferring sea level change and landscape evolution in the Hudson Bay Lowlands, Manitoba, Canada. J. Paleolimnol. 51, 325–341. http://dx.doi.org/10.1007/s10933-013-9714-x.
DIN 38409-H7-2, 2005. German Standard Methods for the Examination of Water, Waste Water and Sludge - Parameters Characterizing Effects and Substances (Group H) - Part 7: Determination of Acid and Base-neutralizing Capacities (H 7). Beuth Verlag, 2005–2012.
DIN 38405-1, 1985. German Standard Methods for the Examination of Water, Waste Water and Sludge; Anions (Group D); Determination of Chloride Ions (D 1). Beuth Verlag, 1885–1912.
DIN EN ISO 11885, 2009. Water Quality - Determination of Selected Elements by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) (ISO 11885:2007); German Version EN ISO 11885.
DIN EN 13946, 2014. Water Quality—guidance Standard for Routine Sampling and Pre-treatment of Benthic Diatoms from Rivers.
DIN EN 14407, 2014. Water Quality–Guidance Standard for the Identification and Enumeration of Benthic Diatom Samples from Rivers, and Their Interpretation. Eur. Stand. EN 2004.
Dudka, S., Adriano, D.C., 1997. Environmental impacts of metal ore mining and processing: a review. J. Environ. Qual. 26, 590–602.
Dufrêne, M., Legendre, P., 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecol. Monogr. 67, 345–366.
Dunlop, J.E., Horrigan, N., McGregor, G., Kefford, B.J., Choy, S., Prasad, R., 2008. Effect of spatial variation on macroinvertebrate salinity tolerance in Eastern Australia: implications for derivation of ecosystem protection trigger values. Environ. Pollut. 151, 621–630.
European Commission, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy. Off. J. Eur. Communities 43, 1–72.
Grantham, T.E., Cañedo-Argüelles, M., Perrée, I., et al., 2012. A mesocosm approach for detecting stream invertebrate community responses to treated wastewater effluent. Environ. Pollut. 160, 95–102.
Gorostiza Langa, S., 2014. Potash extraction and historical environmental conflict in the Bages region (Spain). Investig. geográficas 5–16. http://dx.doi.org/10.14198/INGEO2014.61.01.
Goslee, S.C., Urban, D.L., 2007. The ecodist package for dissimilarity-based analysis of ecological data. J. Stat. Softw. 22, 1–19.
Hartman, K., Kaller, M., Howell, J., Sweka, J., 2005. How much do valley fills influence headwater streams?. Hydrobiologia 532, 91–102. http://dx.doi.org/10.1007/s10750-004-9019-1.
Heckmann, L.-H., Friberg, N., 2005. Macroinvertebrate community response to pulse exposure with the insecticide lambda-cyhalothrin using in-stream mesocosms. Environ. Toxicol. Chem. 24, 582–590. http://dx.doi.org/10.1897/04-117r.1.
Herberich, E., Sikorski, J., Hothorn, T., 2010. A robust procedure for comparing multiple means under heteroscedasticity in unbalanced designs. PLoS One 5, e9788. http://dx.doi.org/10.1371/journal.pone.0009788.
Hofmann, G., 1997. Diatom communities in the rivers Werra and Ulster (Germany) and their response to reduced salinity. Limnologica 27, 77–84.
Hofmann, G., Werum, M., Lange-Bertalot, H., 2013. Diatomeen im Süßwasser-Benthos von Mitteleuropa: Bestimmungsflora Kieselalgen für die ökologische Praxis ; über 700 der häufigsten Arten und ihre Ökologie. Koeltz Scientific Books, 908.
Hudson-Edwards, K.A., Jamieson, H.E., Lottermoser, B.G., 2011. Mine wastes: past, present, future. Elements 7, 375–380.
Hustedt, F., 1953. Die Systematik der Diatomeen in ihren Beziehungen zur Geologie und Ökologie nebst einer Revision des Halobien-Systems. Sven. Bot. Tidskr. 47, 509–519.
Hustedt, F., 1957. Die Diatomeenflora des Fluss-Systems der Weser im Gebiet der Hansestadt Bremen. Abhandl. Naturwiss. Ver. Brem. 34, 181–440.
Jakubick, A.T., McKenna, G., Robertson, A., 2003. Stabilization of tailings deposits: international experience. In: Mining and the Environment III. Canada, Sudbury, Ontario, pp. 1–9.
Johnson, B.R., Weaver, P.C., Nietch, C.T., Lazorchak, J.M., Struewing, K.A., Funk, D.H., 2015. Elevated major ion concentrations inhibit larval mayfly growth and development. Environ. Toxicol. Chem. 34, 167–172.
Kefford, B.J., Buchwalter, D., Cañedo-Argüelles, M., Davis, J., Duncan, R.P., Hoffmann, A., Thompson, R., 2016. Salinized rivers: degraded systems or new habitats for salt-tolerant faunas?. Biol. Lett. 12.
Kefford, B.J., Marchant, R., Schäfer, R.B., Metzeling, L., Dunlop, J.E., Choy, S.C., Goonan, P., 2011. The definition of species richness used by species sensitivity distributions approximates observed effects of salinity on stream macroinvertebrates. Environ. Pollut. 159, 302–310. http://dx.doi.org/10.1016/j.envpol.2010.08.025.
Kefford, B.J., Nugegoda, D., Zalizniak, L., Fields, E.J., Hassell, K.L., 2007. The salinity tolerance of freshwater macroinvertebrate eggs and hatchlings in comparison to their older life-stages: a diversity of responses. Aquat. Ecol 41, 335–348.
Kennedy, A.J., Cherry, D.S., Currie, R.J., 2003. Field and laboratory assessment of a coal processing effluent in the leading creek watershed, Meigs County, Ohio. Arch. Environ. Contam. Toxicol. 44, 324–331. http://dx.doi.org/10.1007/s00244-002-2062-x.
Krammer, K., Lange-Bertalot, H., 1986. Bacillariophyceae 1. Naviculaceae. In: Ettl, H., Gerloff, J., Heyning, H., Mollenhauer, D. (Eds.), Süßwasserflora Mitteleuropa Band 2/1-2/5. Gustav Fischer Verlag, Stuttgart/New York.
Krammer, K., Lange-Bertalot, H., 1988. Bacillariophyceae. 2 Teil. Bacillariaceae, Epithemiaceae, Surirellaceae. Süsswasserflora von Mitteleuropa 2/2. Gustav Fischer Verlag, Jena, 596.
Krammer, K., Lange-Bertalot, H., 1991a. Bacillariophyceae. 3 Teil. Centrales, Fragilariaceae, Eunotiaceae. Süsswasserflora von Mitteleuropa 2/3. Gustav Fischer Verlag, Jena, 576.
Krammer, K., Lange-Bertalot, H., 1991b. Bacillariophyceae. 4 Teil. Achnanthaceae. Kritische Ergänzungen zu Navicula (Lineolatae) und Gomphonema. Süsswasserflora von Mitteleuropa 2/4. Gustav Fischer Verlag, Jena, 437.
Krausmann, F., Gingrich, S., Eisenmenger, N., Erb, K.-H., Haberl, H., Fischer-Kowalski, M., 2009. Growth in global materials use, GDP and population during the 20th century. Ecol. Econ. 68, 2696–2705. http://dx.doi.org/10.1016/j.ecolecon.2009.05.007.
Lawrence, M., 2015. Easy analysis and visualization of factorial experiments. R. package version 4.3. url http://CRAN.R-project.org/package=ez.
Lecointe, C., Coste, M., Prygiel, J., 1993. "Omnidia": software for taxonomy, calculation of diatom indices and inventories management. Hydrobiologia 269, 509–513.
Marshall, N.A., Bailey, P.C.E., 2004. Impact of secondary salinization on freshwater ecosystems: effects of contrasting, experimental, short-term releases of saline wastewater on macroinvertebrates in a lowland stream. Mar. Freshw. Res. 55, 509–523.
Martín-Alonso, J., 1994. Barcelona's water supply improvement: the brine collector of the Llobregat river. Water Sci. Technol. 30, 221–227.
Munné, A., Prat, N., 2009. Use of macroinvertebrate-based multimetric indices for water quality evaluation in Spanish Mediterranean rivers: an intercalibration approach with the IBMWP index. Hydrobiologia 628, 203–225. http://dx.doi.org/10.1007/s10750-009-9757-1.
Ober, J.A., 2016. Mineral commodity Summaries 2016. US Geological Survey.
Odum, E.P., 1984. The mesocosm. Bioscience 34, 558–562.
Otero, N., Soler, A., 2002. Sulphur isotopes as tracers of the influence of potash mining in groundwater salinisation in the Llobregat Basin (NE Spain). Water Res. 36, 3989–4000.
Palmer, M.A., Bernhardt, E.S., Schlesinger, W.H., Eshleman, K.N., Foufoula-Georgiou, E., Hendryx, M.S., Lemly, A.D., Likens, G.E., Loucks, O.L., Power, M.E.,

White, P.S., Wilcock, P.R., 2010. Mountaintop mining consequences. Science (80-.) 327, 148–149.
Pinder, A.M., Halse, S.A., Mcrae, J.M., Shiel, R.J., 2005. Occurrence of aquatic invertebrates of the wheatbelt region of Western Australia in relation to salinity. Hydrobiologia 543, 1–24.
Pond, G., 2010. Patterns of Ephemeroptera taxa loss in Appalachian headwater streams (Kentucky, USA). Hydrobiologia 641, 185–201. http://dx.doi.org/10.1007/s10750-009-0081-6.
Pond, G.J., Passmore, M.E., Pointon, N.D., Felbinger, J.K., Walker, C.A., Krock, K.J.G., Fulton, J.B., Nash, W.L., 2014. Long-term impacts on macroinvertebrates downstream of reclaimed mountaintop mining valley fills in central Appalachia. Environ. Manage 54, 919–933.
Potapova, M., Charles, D.F., 2007. Diatom metrics for monitoring eutrophication in rivers of the United States. Ecol. Indic. 7, 48–70.
Proia, L., Morin, S., Peipoch, M., Romaní, A.M., Sabater, S., 2011. Resistance and recovery of river biofilms receiving short pulses of Triclosan and Diuron. Sci. Total Environ. 409, 3129–3137.
Pudwill, R., Timm, T., 1997. The salinization of streams by water from coal mine spoil dumps in the Ruhr area (Germany). Limnologica 27, 65–75.
R Core Team, 2015. R: a Language and Environment for Statistical Computing. - R Foundation for Statistical Computing.
Reichl, D.I.C., Schatz, M., Zsak, G., 2016. World Mining Data 2016. Minerals Production, Vienna.
Rosenberg, D.M., Resh, V.M., 1993. Freshwater Biomonitoring and Benthic Macroinvertebrates. Chapman & Hall, New York.
Sabater, S., Guasch, H., Marti, E., Armengol, J., Vila, M., Sabater, F., 1992. The Ter, a Mediterranean river system in Spain. Limnetica 8, 141–149.
Sala, M., Faria, M., Sarasúa, I., Barata, C., Bonada, N., Brucet, S., Llenas, L., Ponsá, S., Prat, N., Soares, A.M.V.M., Cañedo-Arguelles, M., **566-567**, 2016 , 1032–1041.. Chloride and sulphate toxicity to Hydropsyche exocellata (Trichoptera, Hydropsychidae): exploring intraspecific variation and sub-lethal endpoints. Sci. Total Environ. .
Schmid, P.E., 1993. Random patch dynamics of larval Chironomidae (Diptera) in the bed sediments of a gravel stream. Freshw. Biol. 30, 239–255.
Schulz, C.-J., 2016. How does salinisation running waters affect aquatic communities? Answers from a case study., in: Drebenstedt, C., Paul, M. (Eds.), Proceedings IMWA 2016, Mining Meets Water – Conflicts and Solutions. Freiberg, Germany, pp. 144–150.
Tachet, H., Richoux, P., Ournaud, M., Usseglio- Polatera, P., 2000. Invertebres d'Eau douce. Systematique, biologie, Ecologie (Freshwater Invertebrates. Taxonomy, Biology, Ecology). CNRS Editions, Paris.
Tokeshi, M., Townsend, C.R., 1987. Random patch formation and weak competition: coexistence in an epiphytic chironomid community. J. Anim. Ecol. 833–845.
Vieira-Lanero, R., 2000. Las larvas de los Tricópteros de Galicia (Insecta: Trichoptera). Universidad de Santiago de Compostela.
Williams, D.D., Hamm, T., 2002. Insect community organisation in estuaries: the role of the physical environment. Ecogr. (Cop.) 25, 372–384.
Zelinka, M., Marvan, P., 1961. Zur Präzisierung der biologischen klassifikation der Reinheit flieβender Gewässer. Arch. Hydrobiol. 57, 389–407.
Ziemann, H., 1971. Die Wirkung des Salzgehaltes auf die Diatomeenflora als Grundlage für die biologische Analyse und Klassifikation der Binnengewässer. Limnologica 8, 505–525.
Ziemann, H., 1999. Salzgehalt. In: Tümpling, W.v., Friedrich, G. (Eds.), Biologische Gewässeruntersuchung. Gustav Fischer Verlag, p. 141ff. Band 2.
Ziemann, H., Kies, L., Schulz, C.-J., 2001. Desalinization of running waters III. Changes in the structure of diatom assemblages caused by a decreasing salt load and changing ion spectra in the river Wipper (Thuringia, Germany). Limnologica 31, 257–280.