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Leaching of two Northern France slag heaps: influence on the surrounding aquatic environment

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Abstract

After the exploitation of coal mines in the 19th and 20th centuries in northern France, many mining slag heaps (SH) were left without any particular management or monitoring. Currently, the influence of these SHs on the quality of surrounding wetlands is hardly known.

The purpose of this work is to determine the water quality in the neighbourhood of two SHs located near the city of Douai and its influence on the distribution of aquatic invertebrates in local wetlands. Our approach involves (1) the spatial and temporal characterization of the water composition (anions, major elements, sulphide, DOC and alkalinity) and of the biological diversity (aquatic invertebrates) and (2), based on this chemical and biological screening, the establishment of relationships between water quality and biodiversity distribution through multivariate data analysis. The results clearly indicate that substantial leaching from the slag heaps occurs, given the very high concentrations of dissolved sulphates (in the range of 2 g L⁻¹). While the pH remains weakly basic, indicating that the leaching water has been neutralized by the highly carbonated regional substratum, high levels of biodegradable organic matter and sulphate contents have been noticed. They sporadically cause significant drops in dissolved oxygen and the occurrence of dissolved sulphides that massively reduce biodiversity, qualitatively and quantitatively. In Summer, oxygen saturation is generally lower due to the higher rate of organic matter degradation, and the risk of anoxic episodes therefore increases. Finally, as wetlands are vulnerable environments, these preliminary results suggest that monitoring and management of these sites must be attempted quickly to avoid the degradation of those valuable habitats.

Synopsis: Leaching of slag heaps introduces in the surrounding wetlands high amounts of sulphates that influences the aquatic biodiversity through redox processes.

Keywords: Slag heap, Wetland, Acid Mine Drainage, Water quality, Biodiversity.

1. Introduction

The Hauts-de-France region faced huge changes between the 17th and the 20th century with the appearance of coal mining which has accelerated its industrial and economic development, but has also transformed its landscape (Foucher et al., 2012). Indeed, the traces left behind by former mining sites are still visible today. They take the shape of hills, called slag heaps (SH), resulting from a long accumulation of mine tailings (mainly black schist and sandstones) (Apourceau-Poly and Bertram, 2016). During this industrial period, the slag heaps were the property of the mining company, Charbonnage de France. However, when the coal extraction stopped, SHs have been rapidly abandoned, leaving in the northern landscape huge piles of natural mining material. Of anthropogenic origin, some of these slag heaps have gradually become green spaces due to a natural return of the vegetation (CPIE Chaîne des terrils et al., 2014). On their slopes and at their feet, they now shelter remarkable but fragile ecosystems. They host unique habitats, thanks to abnormally high temperatures resulting from the lower albedo of the black schist (Denimal, 2001). Hence, they allow rare and protected species to survive in the region and constitute a shelter and/or a feeding place for many others. However, they are highly sensitive to erosion, landslide and leaching. In the early years of this century, these sites have therefore been re-evaluated as Sensitive Natural Areas and have been purchased by the North Department Council, because of their value as biodiversity hotspots (Sader, 2018). Further on, the slag heaps became UNESCO World Heritage in 2012 and are now part of the biodiversity wealth of the region (Apourceau-Poly and Bertram, 2016; Gilbert, 2012). While these slag heaps were thus fostered for their biodiversity wealth, vast wetlands (and more specifically peatlands) spread out in the same area were damaged by the erection of these slag heaps. This created an important environmental problem especially since wetland areas are becoming a worldwide environmental concern: wetland destruction including habitat fragmentation and degradation is increasing while climate change increases the pressure on the

wetland ecosystem (Holland et al., 1995; Davis and Froend, 1999; Gibbs, 2000). They are nowadays facing a strong decline, while they have a proven added value for biodiversity and therefore a need to be protected (Gibbs, 1993; Mitsch and Gosselink, 2000). Wetlands host complex ecosystems including both aquatic and terrestrial biotopes. Moreover, the various habitats, with permanently to temporary water zones, are related to different chemical properties of the water and the soil (Akcil and Koldas, 2006). The story of the slag heaps is thus one with two faces: slag heaps offer a unique environment for many rare species, but their erection has caused habitat fragmentation and degradation of already existing environments.

As mentioned, the SH soils are particularly sensitive to erosion and leaching, either by rainwater or by the oscillation of underground water reservoirs (Figure 1). After such phenomena, the leaching water carries dissolved species and suspended matter from the mining hill into the surrounding wetland and therefore potentially influences this ecosystem (Duffaut, 2001). Slag heap leaching in the Hauts-de-France mine region was initially studied to investigate the impact on groundwater quality (Denimal, 2001). More general studies on post-mining issues included studies on trace metal leaching (Allan, 1995), on Acid Mine Drainage (AMD) issues (Akcil and Koldas, 2006) or on nutrient levels and litter decomposition and their impact on the ecosystem (Lee and Bukaveckas, 2002). The AMD is a common effect of SH leaching which results from the oxidation of pyrite (FeS_2) and produces an acidic leachate source for the environment (Rose and Cravotta III, 1998). Its consequences on aquatic environments such as wetlands are today still poorly studied, specifically in the Hauts-de-France mining area. Moreover, there is a lack of knowledge on the link existing between biodiversity of these wetlands and the leaching inputs of the SHs into them.

Figure 1

Therefore, more studies must be carried out to better understand the diverse influences of SHs on surrounding wetlands and ponds. Invertebrates occupy a central place in aquatic ecosystems since they are ranked at the lower trophic levels of the food chain and they have a role both as consumer (phytoplankton, etc...) and food source (especially for fish and aquatic birds) (Benoit-Chabot, 2014). Information on their nature and quantity is therefore essential for assessing the ecological quality of wetlands at the bottom of slag heaps. Thus, the objectives of the present study are: (i) to establish a state of the art of the physicochemical and biological composition of these atypical environments; (ii) to assess the possible influence of slag heap leaching on the surrounding aquatic ecosystem composition and distribution; and finally (iii) to investigate potential variation of these processes in time and in space.

2. Material and methods

2.1. Study site

Two slag heaps have been selected in agreement with the Northern France Department Council that had noticed recurrent patches of water leaching into the surrounding wetlands. They are both located close to the city of Douai and are called hereafter SH I and SH II (Figure 2). Both have the same tabular shape, but SH I was formed from 1958 to 1984 having a 97-ha surface, while the other one was formed between 1912 and 1975 having a surface of 140 ha (North Department Council, internal communication). The soil surrounding both SHs is mainly occupied by vegetation (agricultural land, woods and meadows) and by water bodies (waterways, ponds and wetlands). The water levels in the ponds and wetlands are relatively stable throughout the year, although they pass through seasonal low and high-water periods. Both SHs are surrounded by a complex network of ponds and wetlands which are fed by the outflow from the slag heaps (enriched in elements from the SH, *i.e.* leaching phenomena) by rainwater and groundwater.

Figure 2

Four very shallow and narrow ponds were sampled at the foot of the SH I (I-01 to I-04) and of the SH II (II-01 to II-06). At the foot of SH II, a deeper and bigger pond (24.5 hectares, 2m deep in average) is also present. Twenty stations were chosen in this pond, but since the results between those stations were not significantly different, only the average of the results is reported in this paper and referred to as station II-07. Finally, a screening of a wetland located in the southern part of SH I was also performed (I-05 to I-15), in order to investigate potential concentration gradients (Figure 2).

Close to a third SH (SH III), a non-impacted reference station, that is recurrently monitored by the Ecology service of the North Department Council, has also been chosen: reference point III-01. According to the internal reports of the Ecology service, the wetland close to SH III is protected from the leaching waters by a clay horizon, resulting in good water quality and good biodiversity indicators (nesting and feeding sites, presence of reed bed, helophytes, hydrophytes, clear water) (North Department Council, 2012). Note further that all GPS coordinates of the sampling points are listed in the supplementary information section.

Two sampling campaigns have been carried out, in order to study possible temporal changes occurring in the environment around the SHs. Each sampling campaign lasted less than 24 h, in order to avoid any influences from weather (rain, drought, etc.) or changes in groundwater levels. A first sampling campaign took place by the end of March 2016 at all 42 stations, including the reference point (III-01). A second one took place by the beginning of July 2016 but only at a few specific stations close to SH I (I-01 to I-04, I-09 to I-12). The second campaign was reduced compared to the first one mostly due to unforeseen events: hunting season and inaccessibility of the sites.

2.2. Sampling, *in situ* measurements and sample pre-treatments

Temperature, pH, dissolved oxygen saturation and conductivity were measured *in situ* at each station using electrodes previously calibrated [pH: combined glass and Ag/AgCl/KCl electrodes (Metter-Toledo), dissolved oxygen saturation: Clark sensor (WTW, Oxi 340) and conductivity (WTW: ProfiLine Cond 3110)]. For the other parameters (alkalinity, anions, major elements, dissolved organic carbon and characterization of organic matter), water was sampled in a clean PFA bottle at about 20 cm depth (when possible) below the surface. A first part of the sample remained unfiltered prior to the biological analysis and was kept at 5°C in an icebox. Back in the laboratory, the aquatic invertebrates were removed from the unfiltered water samples, using a 0.5 mm sieve; aquatic invertebrates were then placed into clean glass flasks, and 70% isopropyl alcohol was added to prevent organisms from decomposing and to avoid predation by higher trophic organisms. The second part of the sample was filtered immediately in the field through 0.45 µm pore size filters (cellulose acetate membranes, Sartorius) for the determination of all parameters, except organic matter. Three aliquots were subsequently prepared for measuring: (i) major elements by addition of 400 µl ultrapure HNO₃ (Fisher Scientific, Optima™); (ii) sulphides by addition of 10 µL of 2N zinc acetate [guaranteed AGR (Analytical Grade Reagents)] and 10 µL of 10⁻² M NaOH (AGR) solution in 10 mL of the sample to stabilize the analyte; and (iii) anions and alkalinity without any treatment but kept at 4°C in an icebox. The third part of the sample was filtered on a glass fibre filter (0.7 µm, Whatmann) previously pyrolyzed at 450°C for 24 hours. This aliquot was kept in the icebox for subsequent analyses of the organic carbon content.

2.3. Chemical analysis

2.3.1. Sulphides

The solution prepared in the field for sulphide measurements was treated by addition of ferric chloride and N,N-dimethyl-p-phenylenediamine oxalate solution to form the methylene blue complex. In order to eliminate the colour due to the presence of ferric ion in the solution,

ammonium phosphate dibasic solution, used as a buffer, is added after 5 min to the last solution (Expertise Center in Environmental Analysis of Quebec, 2015). All the reagents were of analytical grade (AGR). The measurements were performed by spectrophotometry (Varian, Cary 300 Scan UV-Visible) at 664 nm. The sulphide concentration was then determined by comparing the field sample result with that of a calibration curve, made in the same conditions as the field sample. The S(-II) standard mother solution was prepared under nitrogen atmosphere from $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ (Aldrich) and titrated before use by potentiometry with a Cd^{2+} standard solution, using a sulphide ion selective electrode (Orion) and a $\text{Hg}/\text{Hg}_2\text{SO}_4$ reference electrode. The limit of detection of this technique was 0.2 mg L^{-1} and the relative standard deviation carried out on triplicates from several samples was better than 5 %.

2.3.2. Anions

Sulphate, nitrate, chloride, phosphate and fluoride concentrations were assessed with ionic chromatography (Dionex, column Ion Pac AS18, 4x250mm). The MagIC Net 3.1 software was used to process and calibrate the analysis. Limit of detections (LOD) were 0.05 mg L^{-1} for sulphate, 0.01 mg L^{-1} for nitrate, 0.02 mg L^{-1} for chloride, 0.04 mg L^{-1} for phosphate and 0.01 mg L^{-1} for fluoride. The relative standard deviation (RSD) carried out on several triplicate measurements was better than 10 % for all species.

2.3.3. Major Elements

Elemental analyses of Ca, Fe, P, Mn, Mg, Na, Sr, K, Si and B were performed in the water samples by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Varian, Vista Pro, axial view) after multi-elemental External calibrations. Limits of detections (LOD) were 0.1 mg L^{-1} for Ca, 0.06 mg L^{-1} for Fe, 0.2 mg L^{-1} for P, 0.01 mg L^{-1} for Mn, 0.03 mg L^{-1} for Mg, 0.06 mg L^{-1} for Na, 0.01 mg L^{-1} for Sr and 0.1 mg L^{-1} for K. The relative standard deviation (RSD) carried out on triplicates was better than 5% for all species.

2.3.4. Alkalinity

Automatic pH-metric titrations using 0.05 M HCl were carried out (Metrohm, model 848 Titrino Plus) to determine the alkalinity in our samples assuming that, according to pH values (between 6.9 and 8.5) and the other ions present (sulphate, chloride) in the solution, the measured alkalinity is related to the concentration of HCO_3^- . Indeed, at neutral pH, HCO_3^- is the dominant carbonate species and significant contributions of CO_3^{2-} and H_2CO_3 only appear at pH levels greater than 9.0 or lower than 6.0, respectively (Tarvainen et al., 2005). The LOD of this technique is estimated at 0.1 mg L^{-1} (based on a sample volume of 10 mL, a titrant HCl solution at 0.05 M and 50 μL stepwise acid additions) with a relative standard deviation of 5%.

2.3.5. Dissolved Organic Carbon (DOC)

DOC content was analysed using a TOC-meter (Shimadzu, TOC-VCSH), using the NPOC method (Non Purgeable Organic Carbon). An external calibration curve, using potassium hydrogenphthalate, was previously assessed. The LOD is 0.2 mg C L^{-1} with a RSD calculated on several triplicates, lower than 5 %.

2.4. Biological analysis

Two biological indicators (number of aquatic invertebrate taxa and total abundance of aquatic invertebrates) were determined at each station, using 1 L water samples. The aquatic invertebrates have been sorted and then identified through a binocular microscope, using the sight hunting method and determination keys to classify them in a taxonomic family (Tachet et al., 2010). The number of taxa is defined as a group of living beings (here, aquatic invertebrates) from the same family. The abundance was equal to the total number of aquatic invertebrates listed at each sampling station including all taxa.

2.5. Statistical procedures

Principal Component Analysis (PCA) were performed with the R data processing software (www.R-project.org) using two packages: Corrplot and FactoMineR. The analysis of the statistical distributions of the data showed that some parameters were not normally distributed (*i.e.* not following a Gaussian distribution). Spearman correlation and PCA on ranked data were then performed to improve the robustness of the statistical analysis.

2.6. External data

As the groundwaters were not sampled, external data were provided by the French Water Agency from the piezometers 903641, 902687 and 901551 located respectively in Marchiennes, Pecquencourt and Somain (see the Supplementary Information section for the GPS coordinates). These piezometers were selected, because of their proximity to the studied areas.

3. Results and discussions

3.1. General description

pH and conductivity — pH values range from 6.9 to 8.5, including the reference station III-01 with a rather narrow distribution (see Table 1 and Supplementary Information for all data). The conductivity varies from 1.2 to 5.0 mS cm⁻¹ with a median value at 4 mS cm⁻¹ showing a large range of variations. These values, including III-01 (lowest value at 1.2 mS cm⁻¹), are higher than those observed in the surrounding rivers (0.93 mS cm⁻¹ in average) and the groundwaters (0.88 mS cm⁻¹ in average) (Artois-Picardie Water Agency, 2017). This could be due to leaching processes which will be discussed later. But also, as indicated by the value of III-01, this could be due to the higher salt concentrations since evaporation occurs in these small ponds.

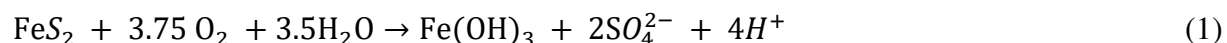
As no threshold values of good status for wetlands have been defined in the European Water Framework Directive, the criteria applied to rivers and ponds (Legifrance, 2015; Official Journal of the European Communities, 2000) was used in this study. Only six sampling points

(I-05, II-07, II-04 II-05, II-06 and III-01) have a conductivity value below the threshold value of 3mS cm⁻¹ indicating that most of the waters are enriched in dissolved salts provided by the SH leaching.

Table 1

Sulphates, cations and alkalinity — Concentrations of sulphate are diverse and range between 900 and 2690 mg L⁻¹ exceeding significantly the bad-status threshold of the WFD (250 mg L⁻¹). These concentrations were also compared to surrounding groundwaters with an average value of 128 mg L⁻¹ (Artois-Picardie Water Agency 2017) (no available data for rivers surrounding). At stations I-01, I-02, I-04, II-01, II-02 and II-03, the sulphate content exceeds 2000 mg L⁻¹, while only at the reference station (III-01) and at the most remote station of SH I (I-05) sulphate concentrations are below 1000 mg L⁻¹.

High sulphate levels are a typical characteristic of SH leaching due to the oxidation of several sulphide minerals like pyrite (Rose and Cravotta III, 1998). When rainwater (oxygenated and with a slightly acidic pH around 5-6) or eventually water from a superficial aquifer encounters the iron sulphide minerals present in the SH, oxidation processes take place allowing the release of H⁺, sulphates and Fe (III). The complete oxidation reaction involved is shown in Equation 1 (Denimal, 2001):

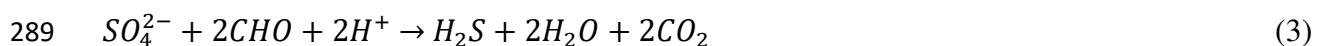


In our case, pH values remain quite neutral and dissolved iron concentrations are low. Indeed, iron levels range from < 0.06 (LOD) to 0.32 mg L⁻¹. This differs from a classic Acid Mine Drainage (AMD) and is called a neutralized AMD (nAMD) (Rose and Cravotta III, 1998). The neutral pH results from the presence of carbonate compounds in the soils (*i.e.* chinks) that buffer the leaching water (*e.g.* calcium carbonate in Equation 2).



Concentrations of calcium, magnesium, potassium, strontium, manganese and sodium vary from 47 to 350 mg L⁻¹ (Ca), 49 to 240 mg L⁻¹ (Mg), 13 to 39 mg L⁻¹ (K), 0.50 to 6.1 mg L⁻¹ (Sr), 0.02 to 1.0 mg L⁻¹ (Mn) and 41 to 480 mg L⁻¹ (Na), showing a wide range of variation and with several values exceeding largely those found at the reference site and in the surrounding groundwater, see Table 1 (no available data for the surrounding rivers). High concentrations of these elements can also be due to slag heap leaching followed by carbonate buffering (nAMD) (Monterroso and Macias, 1998).

A large range of alkalinity values, from 1.51 (II-06) to 24.2 mmol L⁻¹ (I-10), are observed with several values exceeding largely those found at the reference site III-01 (2.76 mmol L⁻¹) and in the surrounding groundwater (5.06 mmol L⁻¹ in average) (no available data for the surrounding rivers). Besides carbonates at circumneutral pH, alkalinity can also include other chemical species such as bisulphide, silicates, borates and phosphates. After quantification of phosphates, of total Si and of B, it is justified to say that, except for station II-01 where sulphides are found at high concentrations (0.9 mmol of sulphides for 2.2 mmol of alkalinity), alkalinity is primarily composed of hydrogen carbonates. Hydrogen carbonate concentration could result from the neutralisation of the AMD, especially at SH II where a significant correlation is observed between alkalinity, sulphate and major cations coming from the leaching (Figure 3). However, for SH I, alkalinity is not correlated with the released species, which can be due to several reasons. Firstly, Rose and Cravotta III (1998) described several neutralisation processes without affecting alkalinity. The acidity can indeed be neutralised by dissolution of FeCO₃ or silicate minerals in which case alkalinity is not affected. Secondly, in SH I and to lesser extent in SH II, the reduction of sulphates by Sulphate Reducing Bacteria (SRB) will consume protons and simultaneously produce gaseous H₂S (Equation 3; Wu et al., 2013).



290 As the dissolved organic carbon concentrations are generally higher in SH I than in SH II, the
 291 bio-reduction of the sulphates may be favoured at SH I, with, as a consequence, the increase in
 292 alkalinity and a poorer correlation between alkalinity and the other slag heap leaching markers.
 293 Thirdly, the non-correlation between alkalinity and the released species may also be linked to
 294 other phenomena like photosynthesis and aerobic respiration, consuming either NO_3^- or NH_4^+
 295 (Stumm and Morgan, 1996). Precipitation of various solid phases, such as metal sulphides or
 296 evaporation when the water level is low, can cause alkalinity changes too.

297 **Anions and DOC** — Nutrient concentrations (NO_3^- and PO_4^{3-}) are possible eutrophication
 298 indicators in aquatic environments. Nitrate levels range from < 0.01 to 0.95 mg L^{-1} , which is
 299 low compared to the surrounding waterbody averages (5 mg L^{-1} for groundwaters, 31 mg L^{-1}
 300 for rivers) and to the WFD threshold value of 10 mg L^{-1} , definition for a “very good state of
 301 water”. Phosphate exhibits very low concentration levels, mostly under the detection limit of
 302 0.04 mg L^{-1} . Except for a few stations, most of them display phosphate levels below the
 303 detection limit. Overall, the impact of urban and agriculture activities on our study sites are
 304 limited and do not favour eutrophication processes in the ponds and wetlands.

305 Fluorides and chlorides can have adverse effects on terrestrial aquatic ecosystems when present
 306 at high concentrations (Elphick et al., 2011; United States Geological Survey, 2009). F^- levels
 307 range from < 0.01 to 0.31 mg L^{-1} , which comply with the average content of the surrounding
 308 groundwater (0.17 mg L^{-1}) and are below the threshold value of 0.5 mg L^{-1} above which F^- has
 309 been shown to affect aquatic organisms (Camargo, 2003). Cl^- concentrations are low (between
 310 8.1 and 26.6 mg L^{-1}) and below the groundwater and III-1 levels (27.1 mg L^{-1}) and the WFD
 311 threshold value of 50 mg L^{-1} (Official Journal of the European Communities, 2000).

DOC concentrations range from 1.6 to 25.1 mg C L⁻¹ with the lowest values at stations I-04 and I-09 and all stations of SH II. At the other SH I stations and at the reference station III-01 (7.5 mg C L⁻¹), the threshold value as defined by the WFD (5 mg C L⁻¹) is exceeded. However, the WFD threshold value for DOC must be handled with caution, because these reference values were originally determined to qualify the state of a water mass (lake, river, etc.), while we are here dealing with wetlands which are, by definition, rich in vegetation and thus in DOC. In a wetland, the WFD threshold value of DOC can be easily exceeded. The highest DOC concentrations were recorded at I-05, I-08 and I-13 with values above 15 mg C L⁻¹, corresponding to a bad status of water bodies. As SH II is far less vegetated than SH I, it is not surprising to find lower DOC values in SH II. This observation can mainly be explained by the difference in age and maturity of both systems. In addition, vegetation maintenance is better in SH II than in SH I where vegetation overgrowth creates obstacles in the water runoff. Finally, as reported by Lee and Bukaveckas (2002), wetlands impacted by mine drainage usually show slower decomposition rates and lower nutrient levels in comparison to wetlands found in predominantly agricultural areas (Lee and Bukaveckas, 2002).

Oxygen, sulphides and total P — Dissolved oxygen saturation ranged from 9 to 140% with an average of about 75%. There is, therefore, a wide range of variation with very high values at some locations (I-01, 02, 03, 05, 09, 12, II-03, 05, 06 and III-01 > 90%) and very low at the surface, even anoxic at the bottom, of others (I-01, 04, II-02 < 30%). Sulphide concentrations were only detected in these three anoxic stations. Total dissolved phosphorus concentrations also seemed to be linked to dissolved oxygen concentration. Varying from < 0.2 to 0.93 mg/L, the variation in phosphate was negatively correlated to that in O₂. It has been observed that in hypoxic or anoxic conditions, the reduction of iron oxides can lead to the release of phosphates in the water that were previously trapped in these oxides (Patrick and Khalid, 1974).

Biological analysis — The biological inventory highlighted the presence of several aquatic invertebrate families. Sixteen families of species, spread over all sampling stations, have been identified and quantified (Table 2), with as the most important, *Cyclops sp*, *Chydoridae* and *Daphniidae*.

The poorest biodiversity levels were observed at SH II stations II-01 and II-02 (respectively, 0 and 1 for the taxon, 0 and 13 for the abundance), where oxygen levels were low and sulphide concentrations high, exceeding 24 mg L⁻¹. Station II-03 showed the highest biodiversity level with 3 taxa and an abundance of 39 individuals. At this station oxygen levels were high, sulphide was not detected, and the highest levels of Ca, K, Mg, Mn and sulphates were observed.

At SH I, the biodiversity wealth seems higher than at SH II: the total number of taxa is 8 (I-01) and the total abundance 288 (I-08). However, large differences between the sampling stations can be observed. The lowest biodiversity levels are observed at stations I-04 and I-05 (respectively, 0 and 2 for the taxon, 0 and 14 for the abundance), the highest levels at I-01 and I-08.

Table 2

Aquatic invertebrate species, also those found here, can be used as bio-indicators of their environment (e.g. Health Monitoring Institute, 2004; Hodgkinson and Jackson, 2005). For instance, *Cyclops sp* (found at all stations except I-04, 06, II-01, 05, 06) and *Diaptomus sp* (found only at II-06) are invertebrate species known for their tolerance to wastewater but also to eutrophication processes. More specifically, *Chironominae* (stations I-01, 03 and II-05), *Ceratopogonidae* (stations I-01 and I-06), *Culicidae* (I-05) and *Nematode* (II-05) are bio-indicators of organic or chemical polluted waters as they are tolerant to such environmental conditions. On the contrary, *Hydrophilus sp* (only found at station I-12) is a species which is

sensitive to water pollution by organic matter and chemicals. *Rotifera* (I-11) is sensitive to salinity, while *Ostracode* (stations I-06, 08, 09, 10, 11, 14) can tolerate salinity but it is sensitive to organic matter, industrial and metal-mining pollution (David M. Rosenberg and Resh, 1993). The salinity resistance of the *Ostracode* family as well as the high alkalinity and the relatively low leaching impact at stations I-06, 08, 09, 10, 11, 14, allowed them to develop there.

3.2. Lixiviation influence

When looking at the results from the PCA (see Fig. 3 – A and C), the first axis with the most variance (PC1 with almost 50% of the data variability) fits well with the lixiviation process. For both sites taken separately, sulphates, major cations, conductivity are close on the right side of PC1. On the opposite side of the PC1 axis, the chlorides are found for both sampling sites (Fig. 3 – A and C) and the DOC is found only at SH I (Fig. 3 – A). These elements are both indicators of non-impacted waters (or less impacted) from the slag heap. A gradient of these two sources is indicated in Figure 3: the first principal component represents the mixing of lixiviates (positive part) and lake water (negative part). The distribution of the points on Fig. 3B gives the relative importance of the two sources for each station. We notice that the “cleanest” stations are in the lakes (I-05 and II-07) and several nearby stations.

Figure 3

The leaching of the slags influences the water composition at the foot of the two SHs. Indeed, sulphates, Ca, Sr, Mg, Na and to a lesser extent alkalinity are correlated at both sites, indicating that all these elements are probably solubilised simultaneously during the leaching process. However, the average composition of the leaching solution after normalisation by the sulphate concentration varies between the 2 SHs as indicated in Table 3. From this table, it is clear that there exists a supplementary mineral phase supplying dissolved sodium and carbonate during the leaching or neutralization process at site I. However, at site II, a slight increase in the

amounts of calcium and strontium can be noticed. The two sites being only 2 km apart, such a difference was surprising. However, it appears that the two slag heaps have different histories. SH I is a relatively recent “washing slag heap”, which was build up between 1950 and 1984 and contains fine particles. SH II is a more historical one (1912-1975), containing a lot of coal and a larger size of slags. In 1975, the remaining coal was extracted from these slags with a washing process and the residues were thereafter redeposited at the same place. Since the mineral composition of the soil between the two sites is different, the lixiviates composition varies as well. It may explain why for SH II, most dissolvable salts, *e.g.* the sodium carbonate phase, may already have been leached.

Table 3

3.3. Hypoxia

Hypoxia or even anoxia has been observed at several sampling sites. This could be due to a complete consumption of dissolved oxygen for the oxidation of organic matter in the small ponds, which are full of vegetal debris. It is also possible that some water leaching from the SH was yet anoxic as all the oxygen was used for pyrite re-oxidation. This is partly supported by the fact that only stations very close to the SH were found with low O₂ concentration and sulphides.

When all oxygen is consumed, anaerobic respiration leads to the reduction of iron oxides and concomitantly the release of P and the reduction of sulphates. The pH also slightly decreases due to the release of CO₂ from oxidised organic matter (Seybold et al., 2002). On the PCA in SH I (Fig. 3A), O₂ and pH are close to each other on the positive part of PC2, but opposite to P and sulphides on the same axis. This is mainly true for SH I. If for SH II the opposition between pH / O₂ and P / sulphides is still visible, it is not as clear as on the second principal component PC2. This lack of complete orthogonality could be the sign of a link between hypoxia and lixiviation on SH II (*e.g.* anoxic lixiviates).

3.4. Chemistry and biodiversity

It seems that for SH I, as it is indicated on Fig. 3.A, lixiviation from the SH and the biological diversity are unrelated. No decrease of the taxon and of the abundance is found along the lixiviation gradient. Lixiviation and aquatic invertebrate diversity and abundance seem orthogonal. Hypoxia seems to influence the invertebrate distribution much more ($R = 0.61$ and 0.55 respectively for O_2 vs taxon and for O_2 vs abundance for all campaigns). The lack of oxygen is very damaging, as it goes pair with the emerging sulphides. The toxicity of sulphides depends on the species, the exposure time and its concentration. Sulphides mainly interfere with respiration metabolism: levels reaching $\mu g L^{-1}$ levels will affect the whole aquatic organism, but concentrations at the $ng L^{-1}$ level are, for instance, enough to impact enzyme functioning like the cytochrome c oxidase which is a transmembrane protein complex and part of the respiratory electron transport chain of eukaryotes including aquatic invertebrates (Castresana et al. 1994). As a competitive inhibitor of the cytochrome c oxidase enzyme, excessive levels of sulphide can lead to cell asphyxia (Grieshaber and Völkel, 1998; Nicholls et al., 2013). Overall, the appearance of sulphides was shown to amplify hypoxia effects (Bagarinao, 1993). The impact and diffusion of sulphide through a cell is also directly proportional to the H_2S concentration in the solution. Indeed, H_2S can easily cross membranes, whereas the hydrogen sulphide HS^- may be electrically excluded. Nevertheless, HS^- can still contribute to toxicity at high concentration (Beauchamp et al., 1984).

For SH II, the quality also depends strongly on the dissolved oxygen concentration ($R = 0.90$ and 0.77 respectively for O_2 vs taxon and for O_2 vs abundance). However, as it has been seen before for this site, hypoxia and lixiviation may not be as independent as it is observed for SH I. Lixiviation from the heap may be indirectly responsible for the lack of invertebrates.

3.5. Temporal variability

In July 2016, eight stations from the first campaign were sampled again: I-01, 02, 03, 04, 09, 10, 11 and 12. Figure 4 compares these results to the ones obtained in March showing the change in wetland composition between these two seasons.

The concentrations of Ca, K, Mg, Mn, Na and Sr drastically decreased for all stations between March and July: a 30% to 70% decrease is systematically recorded (Mg and Sr behaviour is the same as Ca; see Supplementary Information for complete data set). As previously mentioned, these elements are usually carried together with sulphate during the leaching process. However, sulphate concentrations do not really change with time. Complex geochemical processes such as dissolution of gypsum, precipitation reactions of alkaline earth elements with a high amount of carbonates, water exchange between overlying and groundwater must have been involved. These phenomena must have been induced by a lack of precipitation, leaching, temperature increase or water evaporation in the small ponds.

A general decrease of dissolved oxygen saturation and pH, coupled to an increase of alkalinity, was noted. This could indicate that the respiration process exceeded the primary production in these ponds. Indeed, the low water level, the high amount of sediments and particulate organic matter from the microphytes and tree leaves (based on field observations) seem to accelerate the degradation process when the temperature raises.

In parallel, the sulphide concentrations increased at station I-03 and I-12 and decreased at stations I-04 and I-10. We therefore suppose that the reduction of sulphates into sulphides has not been intensified by the increase in temperature and the pluviometry decline. Regarding biodiversity, the number of taxa decreased except at I-09, as well as the abundance except at I-09, I-10 and I-11. Therefore, it seems that the decrease of the biodiversity is linked to degradation processes of organic matter caused by the activity of micro-organisms. These processes reduce the dissolved oxygen concentration and may produce micro-niches of high

dissolved sulphide concentrations. This biogeochemical evolution could therefore lead rapidly to an eutrophication of the water system (Science for Environment Policy, 2015).

Figure 4

4. Conclusion

This study was conducted with the aim of assessing the potential influence of slag heap leaching on the surrounding aquatic environment, in the Hauts-de-France region. We endeavoured to determine the existence or the absence of a link between the chemical composition of surface waters and the biodiversity distribution of each studied zone. One of the main findings relates to the presence of sulphates, which are the first indicators of a mining waste leaching. These sulphate concentrations, which are far above the surrounding background concentrations, are the results of pyrite oxidation contained in the slag heaps soil. Most of the studied areas are enriched in dissolved salts provided by the SH leaching. More precisely, the composition of the water around the two slag heaps reveals a typical example of a neutralized acid mine drainage, with a clear neutral pH and low Fe levels. This was especially confirmed by the presence of carbonate compounds from the soils, high enough to buffer the leaching waters, and by the high concentrations of many typical sub-products of such SH leaching (Ca, Mg, K, Mn, Na). At three sampling stations, located very close to the SH, a reduction of sulphate to sulphide coupled with anoxia and with phosphate release has been evidenced. This phenomenon might be amplified in the case of high amounts of organic matter. According to our results, this has led to a decrease in composition and distribution of aquatic invertebrates, thus in biodiversity. Most likely, the appearance of sulphides (partially under H_2S) intensifies the impact of hypoxia in such ecosystems. Nevertheless, it is difficult to predict whether the ecological imbalance observed at these sampling stations constitutes an isolated case or if it is an indication of what would happen in future on a larger scale in the rest of the wetlands. Despite these three cases, the

invertebrate inventories generally reveal a good biodiversity richness at most of the studied areas: this was mainly linked to good oxygenation conditions of the aquatic environment and to the absence of sulphide. At these stations no influence from SH lixiviation was noticed. From March to July, increasing temperatures coupled with a pluviometry decline do not seem to promote sulphate reduction into sulphide. Even though dissolved oxygen and pH tend to be lower in Summer. Furthermore, loss of biodiversity in July seems only to be related to low dissolved oxygen levels, resulting from higher degradation rates of organic matter by micro-organisms. However, the latter processes may cause, if not controlled and managed, rapid eutrophication of the ecosystem. Finally, the same leaching signature (high sulphate levels, nAMD) is observed in the aquatic environments surrounding both SHs. However, due to a difference in age and maturity between the two SH systems and, to a lesser extent, to a difference in vegetation maintenance, some variations were highlighted, especially regarding DOC levels and the lixivate composition (explained by an historical difference in the SH treatment process).

In future, it would be crucial to continue a scrutiny and scientific monitoring of these very particular and fragile wetlands (including sediments and micro-organisms), especially to better understand their evolution over time during low and high-water periods and during seasonal changes. In addition, monitoring the water fraction migrating to groundwater reserves located beneath the slag heaps would be welcomed as a complementary research approach. Finally, from an operational point of view, it would be necessary to propose remediation actions to prevent possible harmful effects on these wetlands. For instance, more vegetation maintenance around the area and/or the use of pumps could be interesting levers, in order to guarantee a better water circulation, as it was the case in the past during the exploitation of the coalmines.

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Appendix A. Supplementary Information

The Supplementary Information include 4 tables and can be found in the online version.

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639

640

641 **List of figures**

642 **Figure 1:** Overview of the slagheap leaching process (after Thompson, 2015)

643 **Figure 2:** Sampling map for the two slag heaps: SH I and SH II, both close to the city of Douai.

644 SH I is split into 2 distinct zones, which are separated by a significant distance: sampling
645 points I-01 to I-04 are located on the West side of SH I, while points I-05 to I-15 are located
646 on the East side. For confidentiality reasons, the name and aerial views of both slag heaps
647 are not shown here.

648 **Figure 3:** PCA results for site I (A and B) and site II (C and D). Samplings performed in March

649 2016. On site I: A, loadings on the 2 first components and B, scores of the different stations
650 on the same axes. On site II: C and D, same as for site I. S2 represents the sulphides.

651 **Figure 4:** Temporal evolution of the wetland composition between March and July 2016.

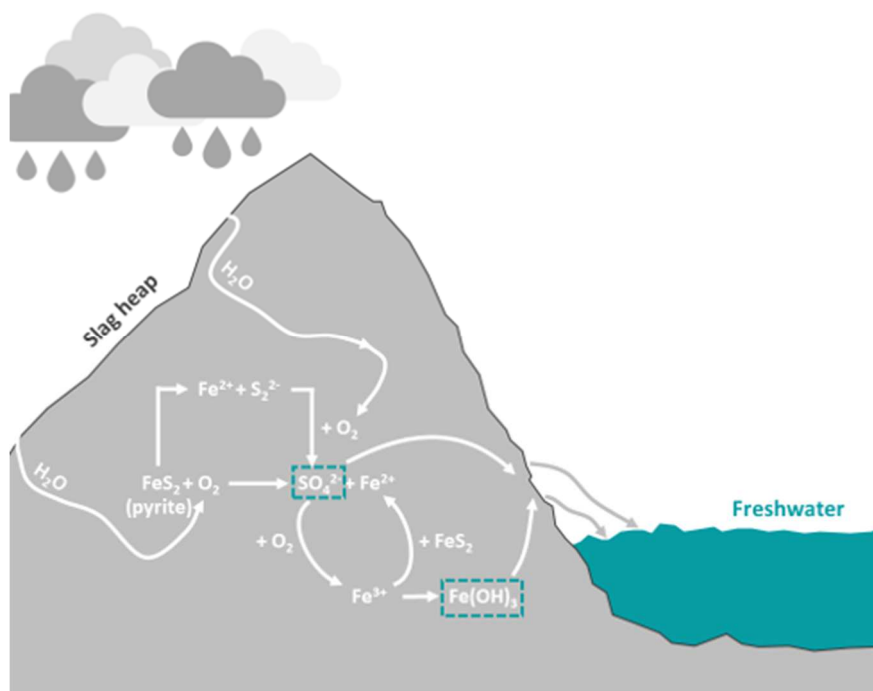


Figure 1: Overview of the slagheap leaching process (after Thompson, 2015)

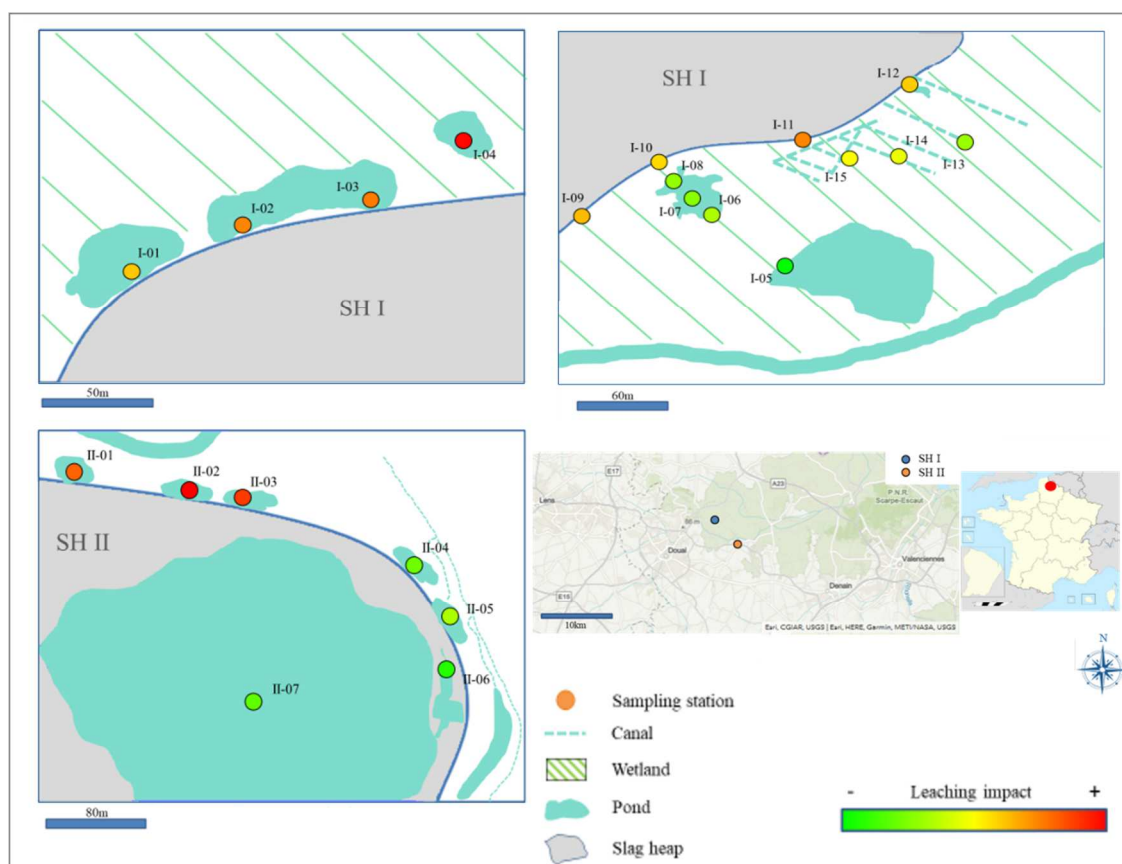


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SH I is split into 2 distinct zones, which are separated by a significant distance: sampling points I-01 to I-04 are located on the West side of SH I, while points I-05 to I-15 are located on the East side. For confidentiality reasons, the name and aerial views of both slag heaps are not shown here.

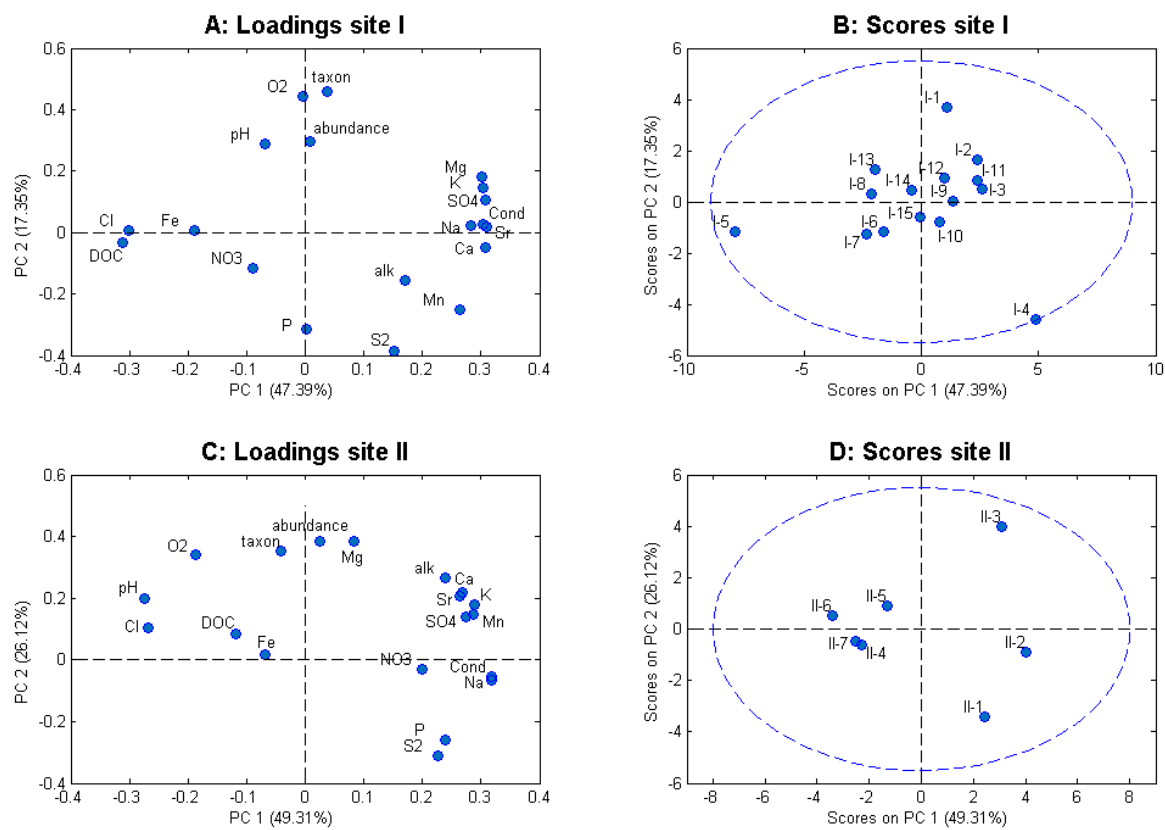


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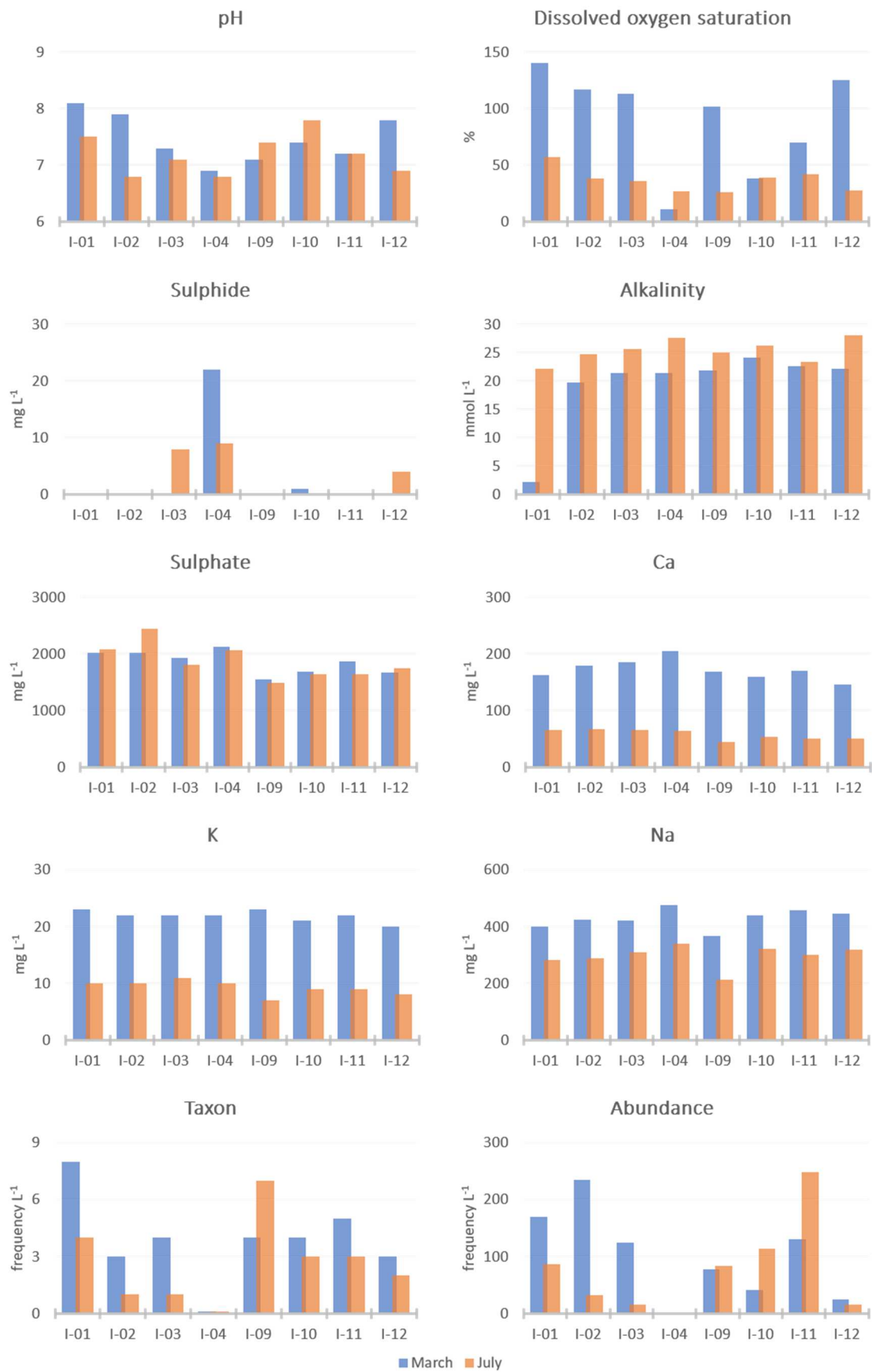


Figure 4: Temporal evolution of the wetland composition between March and July 2016.

List of tables

Table 1: Overview of the water composition for the first campaign (March 2016)

Table 2: Overview of the biological diversity in the water samples. The number of aquatic invertebrates collected in 1 L of water is shown.

Table 3: Sulphate-normalised average concentrations of total dissolved Ca, K, Mg, Na, Sr and alkalinity. * and ** indicates the significance of the ratio difference between site I and site II with a 5% and 1% error respectively.

675 **Table 1:** Overview of the water composition for the first campaign (March 2016)

	min.	max.	mean	reference site III-01	groundwater
<i>In situ measurements</i>					
pH	6.9 (I-04)	8.5 (I-13)	7.7	7.7	7
Conductivity (mS cm ⁻¹)	1.2 (III-01)	5.0 (I-04)	3.6	1.2	0.88
Dissolved oxygen saturation (%)	9 (II-02)	140 (I-01)	75	98	25
<i>Alkalinity (mmol L⁻¹)</i>					
Alkalinity	1.51 (II-06)	24.2 (I-10)	13.7	2.76	5.06
<i>Sulphides (mg L⁻¹)</i>					
Sulphide	< 0.2	30 (II-01)	3.3	< 0.2	-
<i>Dissolved organic matter</i>					
DOC (mg C L ⁻¹)	1.6 (I-04, I-09)	25.1 (I-05)	8.6	7.5	1.6
<i>Major elements (mg L⁻¹)</i>					
Ca	47 (I-05)	350 (II-03)	169	189	147
Mg	49 (I-05)	240 (II-03)	131	60	15
K	13 (I-05)	39 (II-03)	21	2.5	6
Sr	0.5 (I-05)	6.1 (II-02)	2.4	1.1	-
Mn	0.02 (II-06)	1.03 (II-03)	0.29	0.03	0.01
Fe	< 0.06	0.32 (II-04)	0.09	< 0.06	0.03
P	< 0.2	0.93 (II-02)	0.26	< 0.2	-
Na	41 (II-04)	480 (I-04)	307	55	25
<i>Anions (mg L⁻¹)</i>					
NO ₃ ⁻	< 0.50 (I-12, II-06)	0.95 (II-05)	0.68	< 0.50	5
PO ₄ ³⁻	< 0.10	1.17 (II-01)	0.26	0.80	0.03
F ⁻	< 0.01	0.31 (II-05)	0.13	0.21	0.17
SO ₄ ²⁻	899 (I-05)	2693 (II-03)	1732	979	128
Cl ⁻	8.1 (I-09)	26.6 (I-05)	15.0	27.1	27.1

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677

678 **Table 2:** Overview of the biological diversity in the water samples. The number of aquatic
679 invertebrates collected in 1 L of water is shown.

Taxonomic group	SH I										SH II										SH III		
	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	01	02	03	04	05	06	01	
<i>Cyclops sp</i>	69	61	35		13		8	65	28	13	62	12	10	16	7		13	20	19			8	
<i>Diaptomus sp</i>																					10		
<i>Orthoclaadiinae</i>						2		3															
<i>Chironominae</i>	1		2																	3			
<i>Ceratopogonidae</i>	3					3																	
<i>Culicidae</i>					1																		
<i>Chydoridae</i>	30	8						30	15	11	21	12	40	30	11		8		10	6		5	
<i>Daphniidae</i>	62	165	88			80	140	181	33	15	40		61	40	24			11			10	6	
Nematode																				3			
<i>Cyphon sp</i>								1															
<i>Hydrophilus sp</i>												1											
<i>Graptodytes sp</i>	2																						
Collembola	1																						
<i>Rotifera</i>											6												
Ostracode						1		8	2	2	2			7									
<i>Driops sp</i>	1																						
Total taxon	8	3	3	0	2	4	2	6	4	4	5	3	3	4	3	0	1	3	1	3	3	3	
Total abundance	169	234	125	0	14	86	148	288	78	41	131	25	111	93	42	0	13	39	19	16	26	19	

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687 **Table 3:** Sulphate-normalised average concentrations of total dissolved Ca, K, Mg, Na, Sr and
688 alkalinity. * and ** indicates the significance of the ratio difference between site I and site II
689 with a 5% and 1% error respectively.

	SH I	SH II	
Sulphates	1	1	
Ca	0.21	0.26	*
Alkalinity	1.1	0.21	**
K	0.031	0.029	
Mg	0.29	0.33	
Na	1.0	0.26	**
Sr	0.0013	0.0019	**

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