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Environmental characteristics of a tundra river system in Svalbard. Part 2: chemical stress factors

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Abstract: Bacterial communities in the Arctic environment are subject to multiple stress factors, including contaminants, although typically their concentrations are small. The Arctic contamination research has focused on persistent organic pollutants (POPs) because they are bioaccumulative, resistant to degradation and toxic for all organisms. Pollutants have entered the Arctic predominantly by atmospheric and oceanic long-range transport, and this was facilitated by their volatile or semi-volatile properties, while their chemical stability extended their lifetimes following emission. Chemicals present in the Arctic at detectable and quantifiable concentrations testify to their global impact. Chemical contamination may induce serious disorders in the integrity of polar ecosystems influencing the growth of bacterial communities. In this study, the abundance and the types of bacteria in the Arctic freshwater were examined and the microbial characteristics were compared to the amount of potentially harmful chemical compounds in particular elements of the Arctic catchment. The highest concentrations of all determined PAHs were observed in two samples in the vicinity of the estuary both in June and September 2016 and were 1964 ng L^{-1} (R12) and 3901 ng L^{-1} (R13) in June, and 2179 ng L^{-1} (R12) and 1349 ng L^{-1} (R13) in September. Remarkable concentrations of the sum of phenols and formaldehyde were detected also at the outflow of the Revelva river into the sea (R12) and were 0.24 mg L^{-1} in June and 0.35 mg L^{-1} in September 2016. The elevated concentrations of chemical compounds near the estuary suggest a potential impact of the water from the lower tributaries (including the glacier-fed stream measured at R13) or the sea currents and the sea aerosol as pollutant sources. The POPs' degradation at low temperature is not well understood but bacteria capable to degrading such compounds were noted in each sampling point.

Keywords: Arctic, Freshwater contamination, POPs, Bacterial abundance, Bacterial diversity, Environmental changes

1. Introduction

The Arctic contains some highly productive ecosystems despite its extreme environmental conditions, strong seasonal changes in irradiance and snow cover, and the primary productivity concentrated in the short summer (Nguyen et al. 2015). Bacterial extremophiles are among the dominant life forms in the Arctic. They are able to survive in the harsh polar conditions and have developed mechanisms that allow them to cope with a variety of stress factors, e.g. temperature fluctuations, repeated freeze-thaw cycles, high or low levels of salinity or pH, UV light and desiccation (Sahay et al. 2013; Hoover and Pikuta 2013; Ntougias et al. 2016). These environmental stresses are yet enhanced by the increasing concentrations of harmful chemical compounds, including persistent organic pollutants (POPs). There are a few local sources of contaminants in the Arctic, such as military installations, industrial outlets and waste from the old mines, settlements and ships, or the use of insecticides for insect control. However, the majority of Arctic pollution problem arises from a combination of long-range transport of pollutants and the Arctic haze phenomenon, locking the contaminated air in the area for months.

The concentrations of chemical compounds, including contaminants, differ in various aqueous reservoirs: lakes, river and tributaries (Kosek et al. 2018). Pollutants in the environment are exposed to degradative forces. Among them biotic degradation or metabolic processes are known to play a vital role in deciding overall fates of organic pollutants. They not only contribute to the disappearance of the original form of pollutants but also change their physicochemical properties and due to it, their transport and distribution behavior among various compartments in the environment. Physical and chemical factors may render a given contaminant more or less susceptible to bacterial degradation (Matsumura 1989). On the other hand, in aquatic environment, there are some bacterial communities incapable of degrading pollutants, and in such areas the concentration levels of pollutants increase remarkably (Ma et al. 2016; Nadal et al. 2015). Lakes in remote areas such as the Arctic have been of particular interest over the last decade for investigating the fate and dynamics of POPs (Evenset et al. 2004; Evenset et al. 2007; Ahrens et al. 2016). Increasing trends in contamination levels suggest that these areas are significant trapping sites of persistent toxic pollutants (Jiao et al. 2009). Due to the low temperature in the Arctic, mineralization of POPs is extremely slow in cold habitats and they likely bioaccumulate in the adipose tissue and then biomagnify in species inhabiting the polar regions (Kosek et al. 2007). Bacteria inhabiting the Arctic, are more strained and susceptible to the adverse effects of POPs than the bacteria living in other regions due to their long life and slow detoxifying. Moreover, in the nutrient-limited environments, aromatic compounds may serve as a carbon source, also under sulfate-reducing and nitrate-reducing conditions. However, very little is known about their anaerobic degradation pathways (Foght 2008; Mallick et al. 2011), particularly in polar regions. Low temperature catabolic genes/enzymes activity is of a great interest due to the their biotechnological applications.

The main purpose of this article was to study the interactions between the pollutants and bacterial abundance. Selected xenobiotics, such as polycyclic aromatic hydrocarbons (PAHs), phenolic compounds and formaldehyde, and several potentially toxic metals, were determined in the Revelva catchment, as were the bacterial volume and the total number of bacteria. In the selected samples from this river system, metagenomic research was conducted to examine the bacterial community composition and its adaptation to this environment.

2. Materials and Methods

2.1. Fieldwork

The study was conducted in the Revelva catchment (Wedel-Jarlsberg Land, southwestern Spitsbergen, near the Polish Polar Station Hornsund). A detailed map of the sampling area is shown in Part 1 of this article (Kosek et al. submitted) and our former work (Kosek et al. 2018). In brief, the samples were taken from 14 locations in the river, its tributaries and lakes through which it flows, from mountain streams filling rocky beds to its estuary at the Hornsund fjord bay Ariebukta (Table 1). Among the tributaries, the largest one was fed by glacier melt (Ariebekken). Each place was sampled twice, in June and September 2016, reflecting a shift from melting snow patches to permafrost thaw and rainwater as main water sources over the summer season. Furthermore, three points (R4, R8 and R14) were checked for the bacterial taxonomy. The tested points differed remarkably in terms of geological substratum, vegetation and water flow velocity, which influence chemical constituent sources and the potential of self-cleaning for these environments. Points R4 and R8 were located in the areas of no or limited biological soil crust, while the point R14 was surrounded by boggy vegetation, composed of a mixture of mats formed by cyanobacteria and bryophytes, as well as by small lichens and saxifrages in varying proportions (Kumar et al. 2017). Additionally, it should be noted that water was flowing in the points R8 and R14 (most rapidly in R8), while in the point R4 (lake) it was relatively stagnant. Separate aliquots were prepared for chemical composition analysis (in pre-cleaned 1 L HDPE bottles, stored at 4°C), microbiological parameters quantification (50 mL, preserved with 2% formaldehyde, stored at 4°C) and metagenomics (1.5 L, stored frozen).

Table 1. Location of the sampling points in the Revelva catchment in Svalbard.

2.2. Chemical Analysis

The concentrations of PAHs were determined in freshwater samples using Gas Chromatography coupled with Mass Spectrometry Technique, while formaldehyde and the sum of phenols have been determined using Spectrophotometry Method. Trace elements have been determined with Inductively Coupled Plasma Mass Spectrometry. Further technical specifications of the analytical equipment and method, including basic validation parameters of the analytical procedures, are given in Table 2. All blanks were prepared with Milli-Q deionised water. A further chemical description of these samples (inorganic ions, electrical conductivity) can be found in Part 1 of this article (Kosek et al. submitted).

Table 2. Validation parameters and technical specifications used in the applied analytical procedures.

2.3. Quality assurance / Quality Control (QA/QC)

The analytical procedures used to determine individual components in the studied samples have been validated against certified reference materials (CRMs) concordant with ISO Guide 34:2009 and ISO/IEC 17025:2005. The data obtained here were subject to strict QC procedures. The analysis of trace elements involved the application of Standard Reference Material (RM) NIST 1643e Trace Elements in Water, and RM Enviro MAT ES-L-2CRM, ES-H-2 CRM SCP SCIENCE. The calibration of the apparatus was based on RMs by Inorganic ventures ANALITYK: CCS-4, CCS-6, CCS-1, IV-ICPMS-71A. The sensitivity of the applied methods was tested by injecting standard mixtures of the analytes in the measured concentration range. Linear calibration curves of the peak area against standard concentration showed correlation coefficients (R^2) in the range of 0.898–0.999 for all standards. Technically, each sample was analysed in triplicate. The instrumental background was checked by inserting Milli-Q water blanks once per every six samples. All the obtained values for organic compounds (PAHs) in CRMs were within the confidence interval. Reproducibility and recovery for both groups of organic compounds were high (85%–105%) with relative standard deviation (RSD) 4%–10%. Finally, the measurements of formaldehyde and the sum of phenols have been done in accordance with norms ISO 8466-1 and DIN 38402 A51, respectively.

2.4. Bacterial Abundance Analysis

The microbial community parameters quantification has been thoroughly described in Part 1 of this article (Kosek et al. submitted). Briefly, three parameters: total bacterial number, average bacterial cell volume and bacterial biomass (a product of the former two parameters), were quantified in the 28 water samples (14 samples collected in June and 14 samples collected in September). The method applied was epifluorescence microscopy, with DAPI stain, on filters with a pore diameter of 0.2 μm. We used a Nikon Microscope 80i with NIS-

Elements BR 3.0, a MultiScan automated image analysis system, and a high resolution color digital camera (Nikon DS-5Mc-U2).

2.5. Bacterial Community Structure Analysis

In this study, we use the data obtained in Part 1 of this study (Kosek et al. submitted) to analyse another aspect of an Arctic tundra river system: the impact of chemical stress factors on abundance and bacterial community. As a background, we briefly describe the type of data used here and the methods applied in their acquisition. The following paragraph concerns 6 samples in total, collected in points R-4, R-8 and R-14 in June and September. The data was then used with a special focus on bacterial genera which could decompose pollutants, especially from the PAHs group.

The bacterial community structure, i.e. percentage division into main phyla and the smaller taxonomic units (including genus, or even species level, if possible to determine unequivocally), was analysed using next generation sequencing (NGS) technology. This was conducted in 0.2-μm filter residue, from which microbial DNA was isolated and analysed using 16S microbial sequencing on a MiSeq platform (Illumina). Prior to this procedure, the DNA concentration was determined with an ND-1000 UV–Vis spectrophotometer. On the obtained DNA samples, PCR (polymerase chain reaction) was conducted using Q5 Hot Start High Fidelity 2X Master Mix (New England Biolabs), following amplification with the primers: 341F – CCTACGGGNGGCWGCAG and 785R – GACTACHVGGGTATCTAATCC. The results were processed using a set of bioinformatics tools (see Part 1, Kosek et al. submitted). The affinity of the bacterial communities found in the analysed samples was explored with cluster analysis, and these were used for the estimation of biodiversity indices.

3. Results and Discussion

3.1. Chemical stress factors occurring in the studied freshwater samples

3.1.1. pH

In freshwater environments, pH has been shown to be a decisive environmental factor determining the bacterial community composition, often being the most important one compared to factors such as temperature, organic matter, water retention time, and nutrient concentrations (Lindström et al. 2005). pH is also an environmental factor that can vary greatly in aquatic ecosystems (Bååth and Kritzberg 2015). Lake, river and stream waters can have pH values below 4 and above 9 even within small geographical areas. In highly productive lakes, pH at the surface may be 2 units higher than in bottom waters. The variation of the values is driven by vertical differences in photosynthesis, respiration, and redox conditions (Wetzel 2001). pH can also fluctuate rapidly. For example, during snow melt and rain storms, pH values in streams can decrease several units, sometimes within a few hours (Lawrence 2002). On the other hand, sunny days can result in high photosynthetic activity with the increase of water pH values. Accordingly, changes of 2-3 pH units may be found in highly productive aquatic environments (Tank et al. 2009). During episodes of rapid pH changes, the bacterial community may not be optimally adapted to the new pH condition, resulting in impaired functions, and sometimes, inhibition of bacterial growth (Bååth and Kritzberg 2015). Freshwater pH values, and in particular their changes, may pose a big threat to bacterial development and play a key role as a stress factor. However, in our study, the pH values in the collected samples were differing only slightly and ranged from 7.0 to 8.0 both in June and September 2016 (Figure 1). Former hydrochemical studies of the Hornsund fjord area (including Revelva catchment) show high hydrochemical variability, with some values within similar ranges as described in Part 1 of this article (Kosek et al. submitted). Small pH values variation can be explained by the ability of bacteria to regulate the pH of water. Consequently, bacteria are able to survive and develop even in the most harsh conditions. The interaction between the microbes may be set by how their metabolism change the environment and react to those changes. Furthermore, many biochemical reactions involve a turnover of protons and bacteria also alter the pH around them. When the pH modification is beneficial for the bacteria, there is a positive feedback on their growth. The more bacteria there are in the water, the stronger they can change the environment. At adverse pH conditions, a sufficiently high cell density may therefore be needed to survive at all (Ratzke and Gore 2018).

Figure 1. The pH values detected in freshwater samples collected in June and September 2016.

3.1.2. Trace elements

Trace elements were also determined in the samples from both June and September 2016. The concentrations of the following trace elements were determined in them: Li, Be, Al, V, Cr, Mn, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ba, Tl and U (Table 3). The CVs of the obtained results ranged from 0.5 to 1.5 %.

Table 3. Concentrations (±standard deviation, SD) of trace elements in the collected freshwater samples.

The Revelva catchment waters are enriched in trace elements due to the presence of ore-bearing veins and metamorphic rocks in the area (Wojciechowski 1964; Smulikowski 1965). This geological substratum is more exposed in the upper parts of the catchment. Furthermore, the spatial variability of the underlying rocks in this catchment allows for the more abundant occurrence of titanium, possibly also barium, caesium, lithium, rubidium, and zinc in the upper part of the catchment; of zirconium in the left tributaries of the middle part, and of chromium and vanadium in both these areas. The local rocks are relatively abundant in aluminum and manganese throughout the catchment (Smulikowski 1965). As for the ore-bearing veins, in the area occur those with chalcopyrite, cuprite, malachite and azurite, which are copper minerals, as well as smaller concentrations of sphalerite (with zinc) and galena (with lead). The specific locations of these veins favour the occurrence of copper near Skoddefjellet mountain, in the Arie glacier valley, and in the left tributaries of the biggest and smallest lakes in the valley (the top nameless lake and Revvatnet), the occurrence of lead in the Arie glacier valley and of both lead and zinc in the left tributaries of the smallest lake (Wojciechowski, 1964).

The trace metal concentrations detected at the two sampling occasions markedly differed from each other, with an increase in September. This may be caused by the occurrence of groundwater associated with the active layer of permafrost, which gains more importance in the hydrological regime of Revelva as snow patches disappear in the catchment. Apart from the local natural occurrence of trace elements, they are assumed to be derived to the Arctic mostly from long-range atmospheric transport (AMAP 2009), and these may be additionally supplied by September rainfalls. The increase in concentration of trace elements in September 2016 was most evident in the central part of the lake shore and in two points located near the river estuary. The water stagnating in the lake experiences longer contact with suspended mineral matter, hence probably the higher trace element concentrations there. A similarly longer time may have contributed to the higher concentrations near the river mouth. Differences in individual trace element concentrations can be explained qualitatively in terms of mineral surface reactions, complexation, chemical weathering and sorption to solid-phase soil organic matter (Colombo et al. 2018), yet the detailed extent of these processes cannot be determined with the limited data we obtained and it is outside the scope of the current paper.

3.1.3. Organic compounds

In the collected samples, we have determined polycyclic aromatic hydrocarbons. Their concentration levels, as well as those of formaldehyde and the sum of phenols, are reported in Table 4.

Table 4. Concentrations (±standard deviation, SD) of PAHs, formaldehyde and the sum of phenols in the collected freshwater samples.

PAHs are a group of environmentally persistent organic compounds of varied toxicity, usually formed during the incomplete combustion of fossil fuels, biomass, and through other industrial activities. They have been found in the Arctic environment, originating both from the long-range atmospheric transport (Wang et al. 2013) and the local sources. Both human activity and natural phenomena (forest fires, volcanic eruptions) can produce them. PAHs have been found widely in polar environmental media: the atmosphere, water, sediments and biota (Polkowska et al. 2011; Kozak et al. 2017). They can be deposited and accumulated in ice for a long period of time and released to the environment when temperature exceeds the melting point (Ge et al. 2016). The results of PAHs analysis are shown in Table 4. The highest concentrations of PAHs have been detected in the sampling point R13 in June and in the sampling point R8 in September, and these were 1871±59 ng L^{-1} (ANT) and 991±42 ng L^{-1} (FLA), respectively. Comparing the results of PAHs concentrations determined in summer 2016 with those collected and determined in summer 2015, it can be seen that the highest concentrations were observed in the same sampling points (Kosek et al. 2018). Slightly higher concentrations of PAHs were observed in the samples collected in 2016, but the differences are not statistically significant (Kruskal-Wallis ANOVA, all p levels for PAH congeners were above 0.13).

The sampling point R13 is located at the outflow from the Arie glacier, thus such a high concentration of PAHs observed in June can be explained by releasing pollutants from the melting snow cover of the glacier. The difference between PAHs composition in June and September in the catchment (Figure 2) reflects well the order of PAHs elution from melting snowpack and the preferential storage of the more hydrophobic PAHs in ice (Kozioł et al. 2017).

Figure 2. A box - whisker plot of the mean PAHs concentrations in water samples collected in June and September. Significant differences between seasons are indicated with the non-parametric Kruskal-Wallis ANOVA p-levels below 0.05 and given in boldface. The box encompasses the mean value ± SD, the whiskers show the full range of values noted (where <LOD values were assigned a half of the LOD level).

3.2. Microbial community

Various toxic elements and compounds, depending on their concentration in the environment and simultaneous effects of their occurrence, may or may not be an effective inhibitor of bacterial growth in aquatic environments. In Part 1 of this article (Kosek et al. submitted) we report bacterial abundance indices (total bacterial number, average cell volume and bacterial biomass) in the collected samples. Both spatially and temporally, these indices were characterised by a pronounced variability.

For this reason, in this study in June and September 2016, three points (R4, R8 and R14) were checked for the bacterial taxonomy. The general structure of studied microbial communities (based on the relative abundance of classified sequences) was composed mainly of bacterial taxa, with 43-53% *Proteobacteria*, 9-23% *Actinobacteria*, 6-12% *Bacteroidetes*, and more than 2% of *Planctomycetes, Firmicutes* and *Verrucomicrobia* in all samples (Part 1, Kosek et al. submitted). Interestingly, *Proteobacteria*, *Bacteroidetes, Firmicutes*, and *Actinobacteria* were also identified as the core phyla in activated sludge of municipal and industrial wastewater treatment system (Ibarbalz et al. 2013), which are typically polluted waters.

Indeed, some of the bacterial strains found in the sampled waters may be capable of decomposing specific pollutants. The specific allochthonous organic compounds of potential toxicity may modify the taxonomical structure of the microbial community and lead to the selection of bacteria that are capable of metabolizing them. For example, we have detected bacteria from *Flavobacteriaceae* (Bacteroidetes phylum) family, which are linked to the degradation of PAHs at low temperature (Eriksson et al. 2003). However, in the environmental niches, to obtain complete degradation of organic compounds, usually the complex bacterial community is involved. Therefore, in future studies it would be valuable to combine the data obtained from NGS with the analyses of specific functional genes involved in particular PAHs degradation.

Up to now numerous unique metabolic pathways of PAHs biodegradation have been already documented (Peng et al. 2008; Mallick et al. 2011; Ghosal et al. 2016), but in this terms the knowledge on the bacterioplankton community inhabiting the inland water system of the polar area is limited. Additionally, many of the reported data were from incubation of single or mixed cultures in laboratory experiment, while for in situ consortia and the observed for them degradation potential may differ, due to the combined activities of whole community members.

Major PAHs degrade approaches are highly linked to the oxygen presence/absence. Under aerobic conditions the oxygen is both the final electron acceptor and co-substrate to activate and subsequently cleave the aromatic ring, catalyzed by oxygenase enzymes (monooxygenase or dioxygenase) (Foght 2008; Carmona et al. 2009). In the anoxic conditions, which is regarded as more common in natural environments (e.g. aquifers, aquatic sediments and submerged soils), the attack on the aromatic ring is primarily based on reductive reactions (Foght 2008; Carmona et al. 2009), where nitrate, sulfate or ferric ions are used as final electron acceptors (Foght 2008; Carmona et al. 2009). For instance, *Hyphomonas* detected in each sampling point in this study (see Table 5), showed the degradation potential after the addition of sources of nitrogen and phosphate to hydrocarbon-contaminated harbour sediments (Yakimov et al. 2005). The metabolic pathways of sulfate-reducing and Fe(III)-reducing bacteria are also of interest due to the role they play in the biogeochemistry including degradation of organic contaminants (Meckenstock et al**.** 2000). The sulfate-reducing *Desulfovibrio*-like bacteria, forming up to 0.26% of the bacterial community composition in the studied samples, as well as the spore-forming *Desulfotomaculum* and *Desulfosporosinus* genera from *Firmicutes* phylum (forming up to 0.22 % of the bacterial community), are also potentially capable of decomposing the organic pollutants occurring in this catchment, since these bacterial groups may use a variety of aliphatic and aromatic compounds as a carbon source (Hansen 1994). Interesting is also high relative abundance of psychrotolerant *Rhodoferax* genus (from 1.56% to 5.6%), already reported as potential phenanthrene degrader (Martin et al., 2012), and in this study represented mainly by *R. ferrireducens* (>85%), which is capable of dissimilatory Fe(III) reduction at low temperature.

Additionally low molecular weight (LMW) PAHs, as naphthalene, anthracene and phenanthrene, more volatile and soluble in water are also more susceptible to biodegradation then high molecular weight (HMW) PAHs, thus also select for different microbial consortia (Vila et al. 2010). Some reports indicated the catabolic versatility of some bacteria (e.g. *Pseudomonadales* and *Sphingomonadales* from *Gammaproteobacteria* and *Alphaproteobacteria* classes, respectively) is linked to the presence of plasmid-encoded aromatic degradative genes (Peng et al. 2008), which can be disseminated by horizontal gene transfer to phylogenetically diverse bacteria (Nojiri et al. 2004). The list of members of bacterial community detected in studied sampling points and possibly involved in the pollutants degradation is given in Table 5.

Table 5. The list of members of bacterial community possibly involved in the degradation of pollutants. A genus was considered only if it constituted ≥ 0.01% of the community from a single sample.

Expanding the information on the variety of bacterial phyla and metabolic pathways presented in Part 1 of this article (Kosek et al. submitted), we conclude that various organic compounds (including those considered pollutants) may be also decomposed by them. This is consistent with the detection of pollutant-decomposing bacteria in such remote parts of the Arctic as the surface of the Greenland Ice Sheet (Hauptmann et al. 2017).

3.3. Statistical analysis of bacterial abundance and chemical background in the Revelva catchment

A Principal Component Analysis (PCA) was performed to encompass the wider set of chemical variables connected to potentially toxicity (PAHs, HCHO, phenols and trace elements such as Ni, Zn, Cu, Co, Be, As, Mn, as well as pH, the extreme values of which are also a sign of hospitable environments). The above-mentioned trace elements were chosen based on the literature review by (Kabata-Pendias and Pendias 2001), on the basis of their highest potential for toxicity in the general biosphere (especially for plants). The quantitative data on the bacterial community, such as the total bacterial number (TBN) and the average volume of bacterial cells (ACV) described in details in Part 1 of this article (Kosek et al. submitted), were included in the analysis. For brevity, each PAH is referred to by an abbreviation listed in Table 2 of this manuscript. The PCA for this study was performed (Figure 3, 4) using R v. 3.4.4, using the *prcomp* function, on a log-transformed dataset, except the pH value which is a logarithm.

In the analysis conducted for the whole summer season, the scree plot shape suggested that the PCs 1, 2 and 3 were likely significant factors. We also conducted further analyses for June and September separately, showing only the first two PCs in each (also as suggested by the scree plot)

Figure 3. PCA conducted for the potentially toxic chemicals and indices of the bacterial community structure detected in the hydrological environment of the Revelva catchment. Top: The space defined by the PCs 1 and 2, with a division by the month of sampling (colours). Bottom: Same analysis, space defined by the PCs 2 and 3, division by the type of the hydrological environment. Numbers 1-14 depict samples from locations R1-14 in June, numbers 15-28 denotes samples from the same locations in September, in the same order (e.g. number 28 is the sample from point R14 in September).

A clear division between sampling times was found to be depicted by the two main variability components (PC 1 and 2; Figure 3 Top), which accounted for approximately 34% of the total variability in the dataset. In the beginning of the summer season, elevated concentrations of NAP, ACE, ACY (lower molecular weight PAHs, also more water soluble), HCHO and Zn were noted, as well as a higher ACV bacterial community index. These factors may be linked to the still melting snow patches in the catchment (water-soluble PAHs elute from snowpack earlier, while the other PAHs may be stored as particle-bound and even incorporated into ice by refreezing – Meyer et al. 2009; Kozioł et al. 2017). HCHO may even be produced in snowpack by photochemical reactions of more complex organic compounds, including those occurring in particulate forms (Sumner and Shepson 1999; Grannas et al. 2004). Snow is a medium poor in nutrients, hence the specialised k-strategist bacteria may predominate there, growing but not multiplying rapidly (compare Part 1, Kosek et al. submitted).

On the other hand, the late season was characterised by a higher concentration of most other toxic elements and compounds and the TBN index, which shows that the r-strategists were not limited by these toxic chemicals in their reproduction. This can be interpreted in terms of the concentrations being too low to have a limiting impact on the bacterial community. Furthermore, some of the detected trace elements (such as Cu, Mn and Zn) have ample local sources in the geological substratum and therefore the local bacterial community is well adjusted to their presence. It is interesting to notice, however, that the most abundant presence of zinc deviates from its naturally enriched area in the upper part of the catchment (with higher concentrations in some of the samples located in the lower parts of the catchment, especially points R10 and R13), which may suggest its pollution origin. It is also the only trace metal in this analysis to correlate closer with ACV than TBN.

Multiple further trace elements, as well as phenols and higher molecular weight PAHs, tend to increase in concentration over the course of the summer season, which may be linked to the shift in the hydrological regime from snow-fed to permafrost thaw and occasional rainfall (Pulina et al. 1984). Among the trace elements analysed here, As, Co, Cu, Mn, Ni and Zn have been found by Kozak et al. (2015) in the local rainfall composition, likely representing both the local and distant sources, including rock dust, sea spray and human-activity-related emissions. Especially the Zn concentrations in rainfall may be very high in this region (mean concentration in Kozak et al's study reaching 28.99 μgL^{-1}), and hence it can be treated as a pollutant. Phenols may originate from local plant tissue decomposition (Grannas et al. 2004), which agrees well with their high concentrations in the samples from the lower part of the catchment, where lush tundra vegetation grows. The high-molecular-weight PAHs distinguish the upper part of the catchment, where they could have been stored longer from the snowpack sources, e.g. frozen in the ground ice.

The type of hydrological environment was best distinguished on the PC 2 and 3 graph (Figure 3. Bottom), depicting approximately 27% of the total variability in the dataset. The river samples may have been grouped due to their location in the lower part of the catchment rather than by their difference from the smaller streams, since they are distinguished by the presence of the earlier mentioned phenols, which may come from tundra vegetation, and Cu, Ni and Co, of which at least Cu should occur abundantly in the left tributaries of the lower part of the catchment. The lake samples clustered around such characteristics as higher molecular weight PAHs, some trace metals, HCHO and ACV, which indicates that in certain circumstances the stagnant water may gain more toxic characteristics and be less habitable to the generalist bacteria population, although the effect is not consistent across all samples.

Figure 4. PCA conducted for the potentially toxic chemicals detected in the different hydrological environments of the Revelva catchment (denoted by colour coding) and indices of the bacterial community structure in these waters. Each graph for a separate sampling occasion, the space defined by the PCs 1 and 2. Top: June. Bottom: September. Numbers 1-14 depict samples from locations R1-14 in that particular month.

In June, a similar separate graph was prepared, concentrating on 47% of the total data variability depicted by PCs 1 and 2 (Figure 4. Top). The first PC, explaining almost 32% of the variability, differentiated strongly between the samples with the high and the low concentrations of PAHs, highlighting especially their elevated concentration in the glacier-fed stream at R13. It also maintained the clear division between environments with the high ACV (characteristic for k-strategists) and high TBN (r-strategy indicator; compare Part 1, Kosek et al. submitted). TBN was correlated relatively closely (and positively) with PAHs concentrations in June, hence probably this type of POPs was not counteracting the multiplication of bacteria, and perhaps these compounds could even be used as an organic substrate by some of the organisms present there. However, in September (Figure 4. Bottom), the concentrations of selected PAHs (ANT, FL) and formaldehyde showed a close affinity to ACV, as did Be and As. These compounds may have been limiting factors to bacterial multiplication in the samples taken in the upper part of the catchment then. However, TBN remained in a positive correlation with NAP and phenols.

4. Final remarks and conclusions

The Arctic environment, although it seems to be free from anthropogenic pollution, is not as pristine as it might seem. Remarkable concentration levels of persistent organic pollutants have been detected in the freshwater samples collected from the Revelva catchment. Globally emitted contaminants accumulate in the Arctic and can be stored in this cold environment for a long period of time (Mackay and Wania, 1995; Friedman and Selin, 2016). Moreover, climate change influences the release of these contaminants through elevated melt rates, resulting in increased contamination locally (Blais et al. 2001; Miner et al. 2018). The microbial community interacts with contamination in the Arctic (e.g. Hauptmann et al. 2017), however it is yet unknown how universal such interactions are. The important issue is to know whether contaminants present in the environment are a toxic factor for bacteria, or whether they show the ability to deal with these pollutants and reproduce in spite of their presence. Described research shows that the catchment chosen for this study constitutes a place of accumulation of persistent organic pollutants and also some trace elements that may be toxic for the bacteria. The determined pollutants may pose a serious threat to the development of bacteria in the Revelva catchment. Depending on their concentration in the environment and simultaneous effects of their occurrence, they may or may not be an effective inhibitor of bacterial growth in aquatic environments. However, despite the presence of contaminants and the limited nutrient supply (described in Part 1, Kosek et al. submitted), the Revelva catchment is characterised by a great biodiversity. The general structure of these microbial communities was composed mainly of bacterial taxa, such as *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, *Firmicutes* and *Verrucomicrobia*. The bacterial ability to degrade toxic compounds depends on numerous factors, which were not studied in this research, but there is a possibility that the bacteria present there decompose the described contaminants. For example, *Bacteroidetes* are linked to the degradation of PAHs at low temperature. Denitrifiers, as relatively abundant in this study *Flavobacterium*, sulphur (*Desulfovibrio*, *Desulfotomaculum*, *Desulfosporosinus*) or psychrotolerant Fe-reducing bacteria (*Rhodoferax*) are also potentially capable of decomposing of the persistent organic pollutants occurring in this catchment (Martin et al. 2012; Kappell et al. 2014). The potential for biodegradation of polycyclic aromatic hydrocarbons (PAHs) at low temperature is not well understood, but such biodegradation would be very useful for remediation of polluted sites. Bacteria inhabiting the Revelva catchment have adapted to live in difficult conditions. It can be hypothesised that they show the potential for decomposing persistent organic pollutants, but in order to confirm it, it is necessary to conduct more thorough research at the molecular level.

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Figures

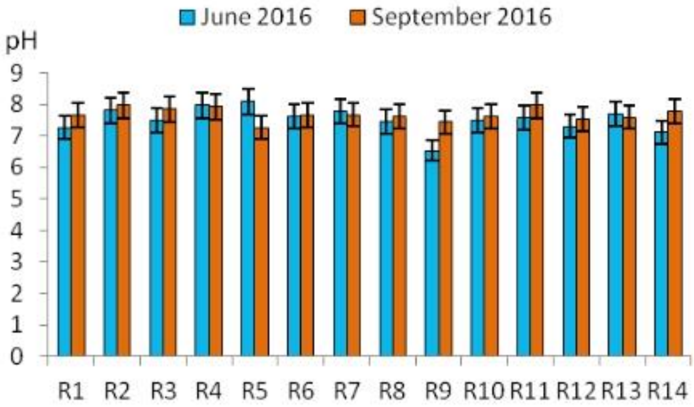


Figure 1. The pH values detected in freshwater samples collected in June and September 2016.

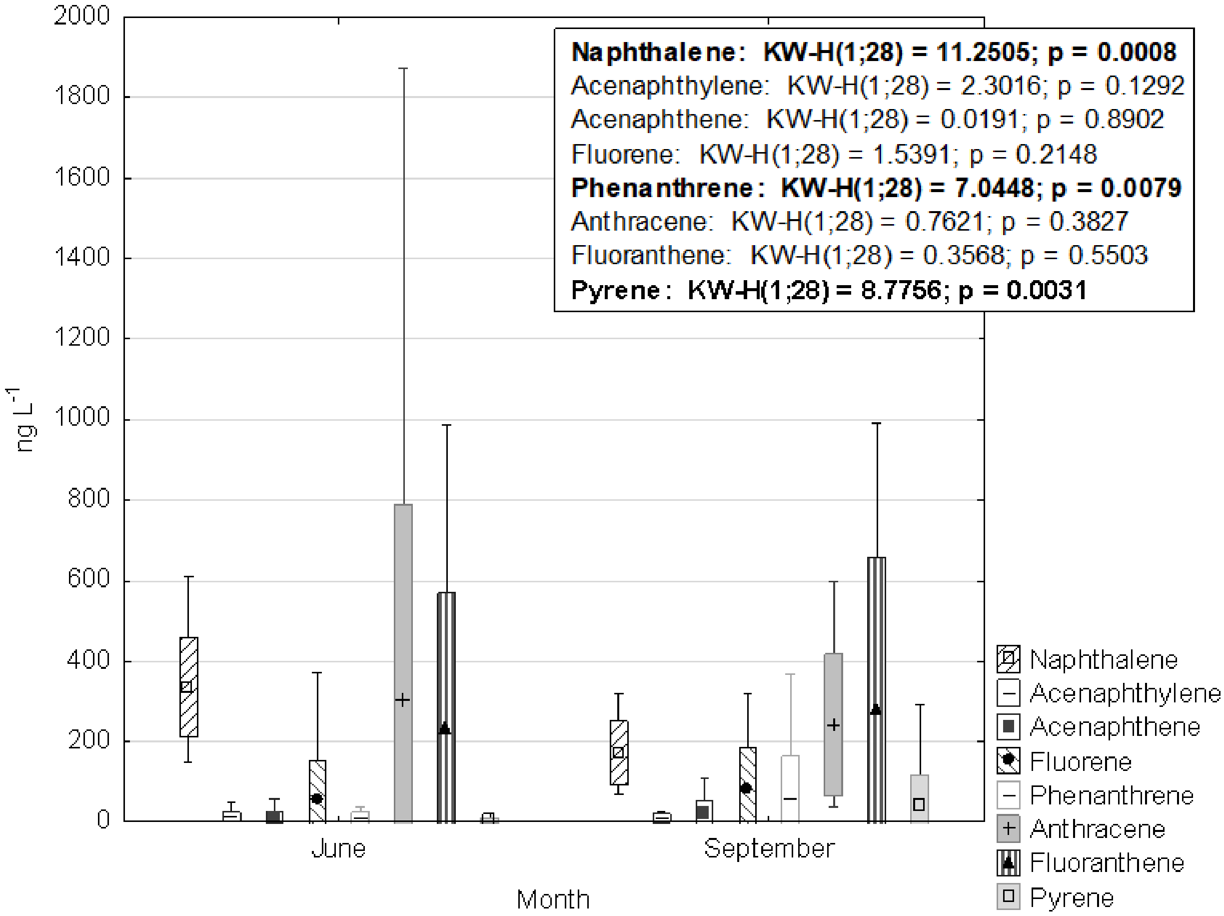
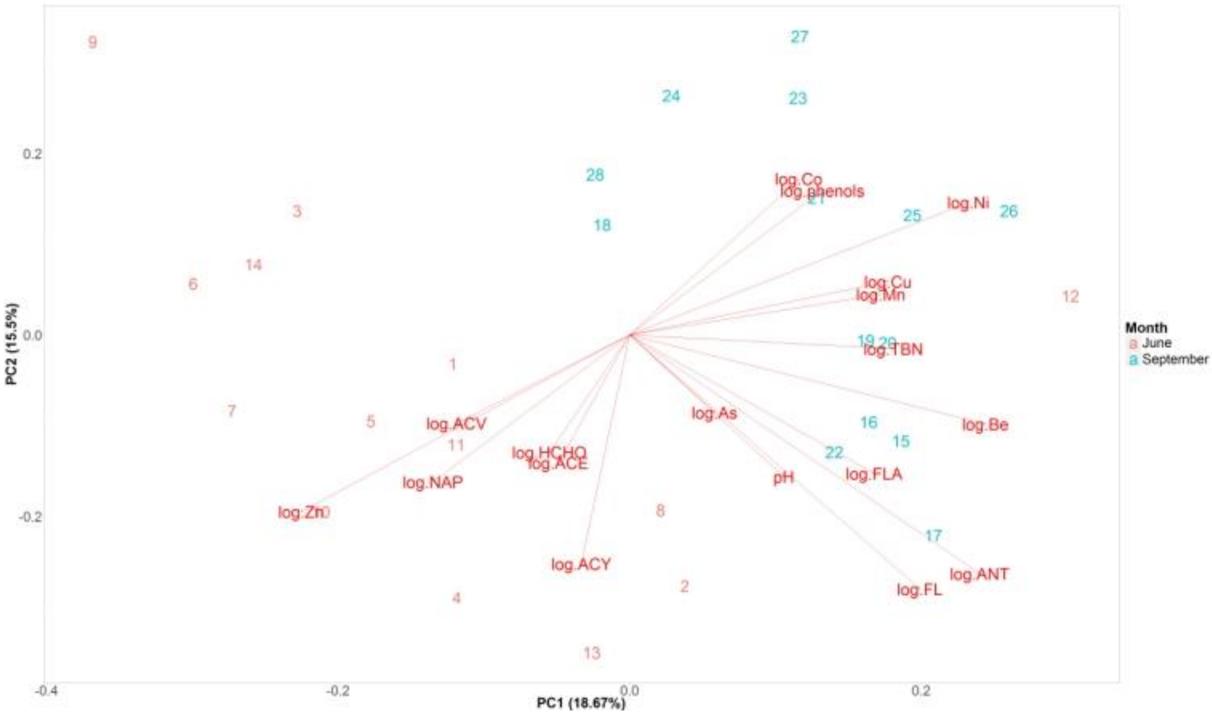


Figure 2. A box - whisker plot of the mean PAHs concentrations in water samples collected in June and September 2016. Significant differences between seasons are indicated with the non-parametric Kruskal-Wallis ANOVA p-levels below 0.05 and given in boldface. The box encompasses the mean value ± SD, the whiskers show the full range of values noted (where <LOD values were assigned a half of the LOD level).



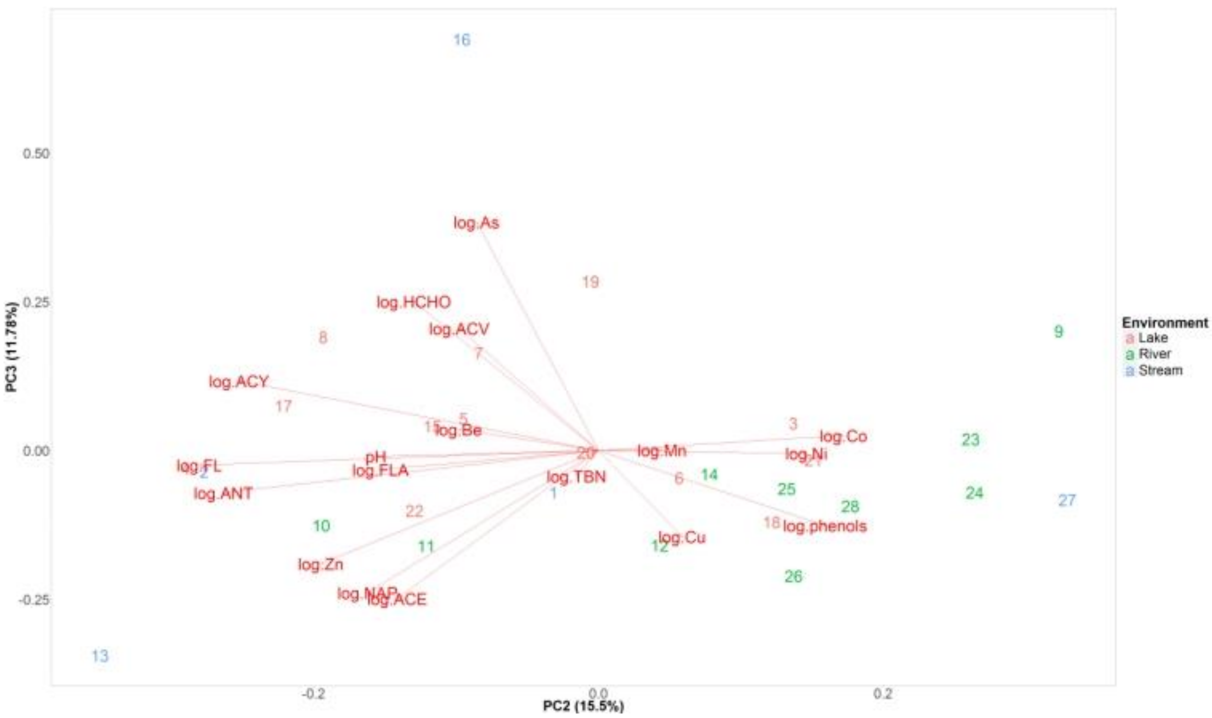
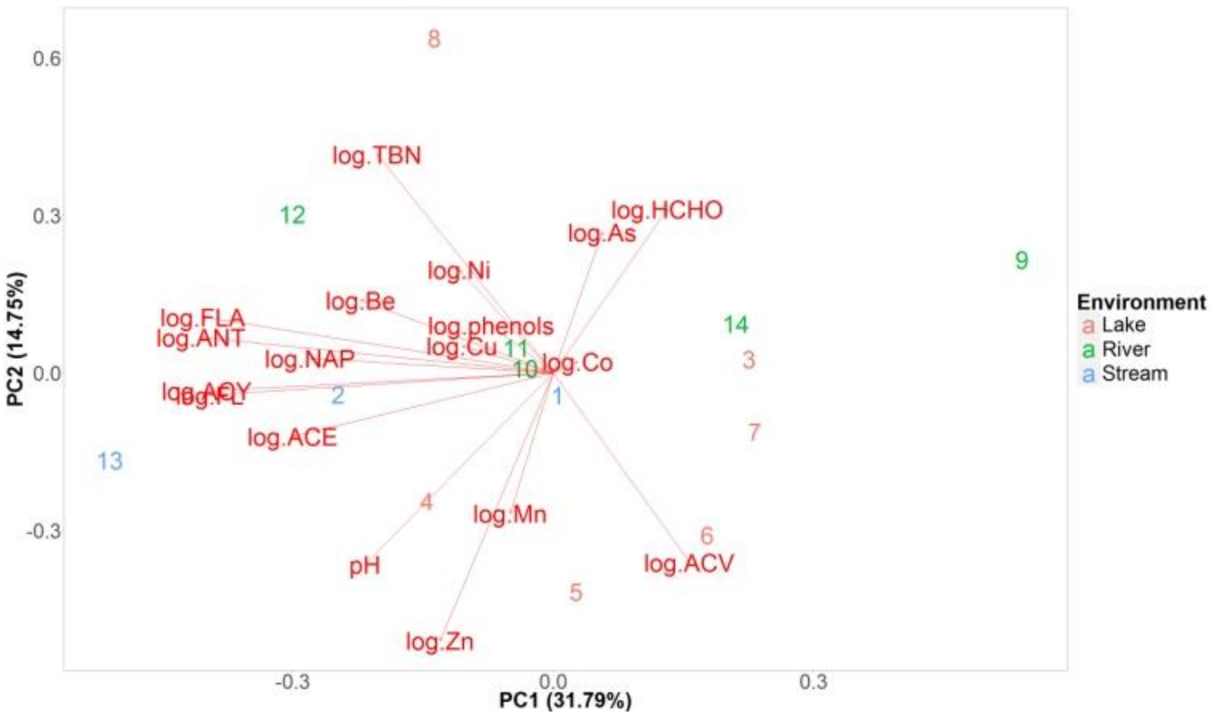


Figure 3. PCA conducted for the potentially toxic chemicals and indices of the bacterial community structure detected in the hydrological environment of the Revelva catchment. Top: The space defined by the PCs 1 and 2, with a division by the month of sampling (colours). Bottom: Same analysis, space defined by the PCs 2 and 3, division by the type of the hydrological environment. Numbers 1-14 depict samples from locations R1-14 in June, numbers 15-28 denotes samples from the same locations in September, in the same order (e.g. number 28 is the sample from point R14 in September).



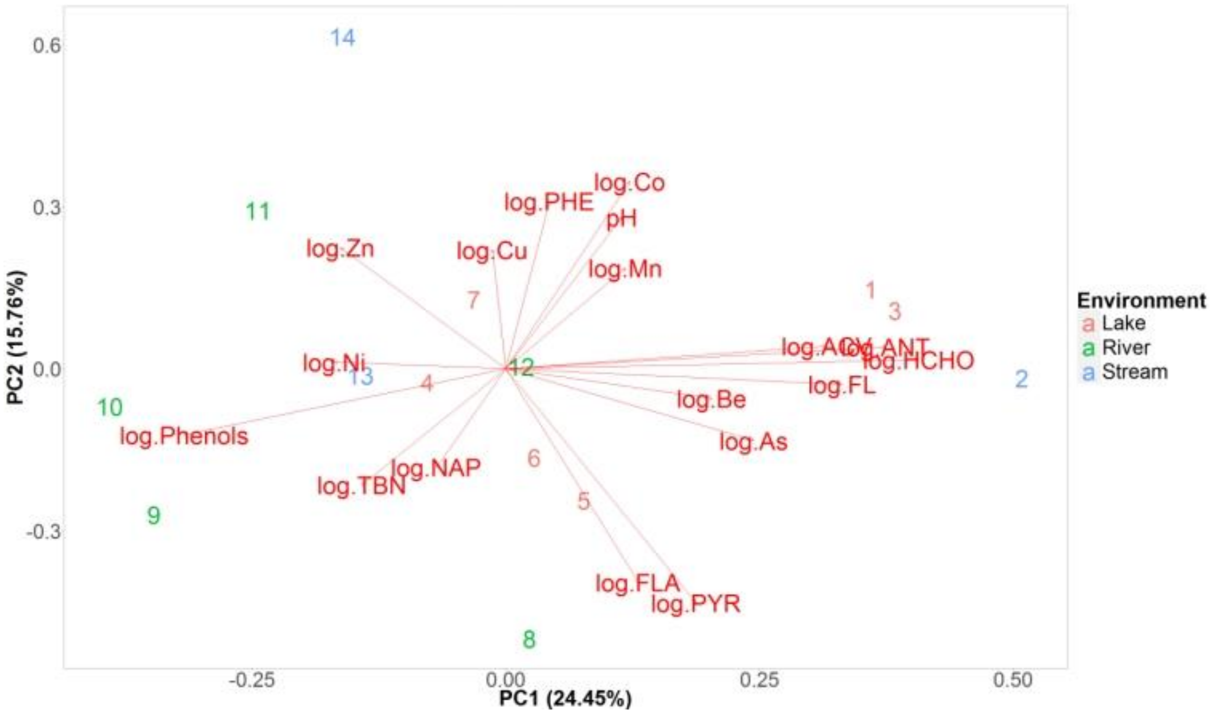


Figure 4. PCA conducted for the potentially toxic chemicals detected in the different hydrological environments of the Revelva catchment (denoted by colour coding) and indices of the bacterial community structure in these waters. Each graph for a separate sampling occasion, the space defined by the PCs 1 and 2. Top: June. Bottom: September. Numbers 1-14 depict samples from locations R1-14 in that particular month.

Tables

Table 1. Location of the sampling points in the Revelva catchment in Svalbard.

ID	Latitude	Longitude
R1	77° 02,174' N	15° 20,391' E
R2	77° 02,170' N	15° 21,021' E
R3	77° 02,113' N	15° 20,391' E
R4	77° 01,960' N	15° 21,282' E
R5	77° 01,437' N	15° 22,505' E
R6	77° 01,218' N	15° 23,385' E
R7	77° 01,122' N	15° 23,690' E
R8	77° 01,022' N	15° 24,077' E
R9	77° 00,841' N	15° 25,028' E
R10	77° 00,949' N	15° 24,686' E
R11	77° 00,640' N	15° 25,905' E
R12	77° 00,040' N	15° 26,675' E
R13	77° 00,332' N	15° 27,209' E
R14	77° 00,179' N	15° 26,902' E

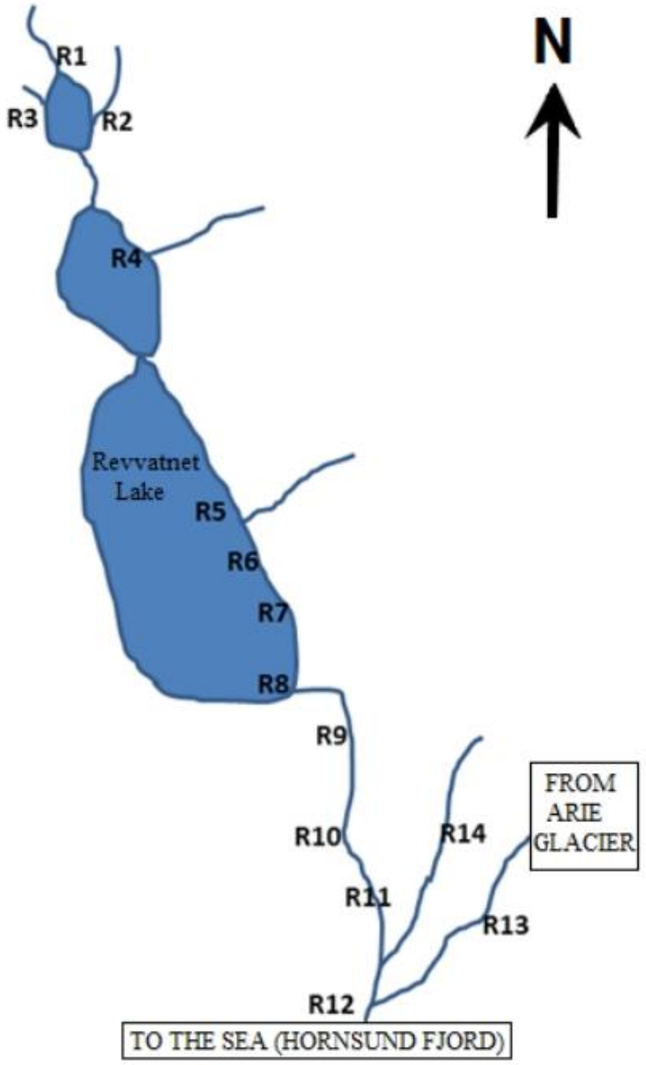


Table 2. Validation parameters and technical specifications used in the applied analytical procedures.

Determined compounds/parameters		Measurement range	LOD[4]	LOQ[4]	Measurement method/technique
pH		-	-	-	Electrochemical method: microcomputer pH-meter(Elmetron), electrode type EPS-1
∑ Phenols[1]		0.025-5.00	0.001	0.003	Spectrophotometry method;
Formaldehyde[1]		0.020-8.00	0.005	0.015	Spectrophotometer 6300, Jenway
PAHs[2]	Naphthalene (NAP)	1.02-3500	0.034	1.02	Gas Chromatography technique coupled with Mass Spectrometry;
	Acenaphthylene (ACY)	0.012-1000	0.004	0.012	Gas Chromatograph 7890A (Agilent Technologies) with the application of Mass Spectrometer (5975C inert MSD Agilent Technologies), detector (Agilent Technologies 5975C) with electron ionization
	Acenaphthene (ACE)	0.012-1000	0.004	0.012	
	Fluorene (FL)	0.005-1000	0.002	0.005	
	Phenanthrene (PHE)	0.008-1000	0.003	0.008	
	Anthracene (ANT)	0.023-1000	0.008	0.023	
	Fluoranthene (FLA)	0.042-1000	0.014	0.042	

	Pyrene (PYR)	0.084-1000	0.028	0.084	
Trace elements[3]	**Li, Be, Ga, Rb, U, Tl, V, Cr, Mn, Co, Ni**	0.010-1000	0.010	0.030	Inductively Coupled Plasma Mass Spectrometry technique; (Thermo Scientific XSERIES 2 ICP-MS)
	Al, Cu, Zn, As, Ba	0.100-1000	0.100	0.300	
	Sr	1.00-1000	1.00	3.00	

[1][mg L^{-1}], [2][ng L^{-1}],[3][μg L^{-1}],[4]the limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the response (s) and the slope of the calibration curve (b), according to the formulas: LOD=3.3(s/b), LOQ=10(s/b)

Table 3. Concentrations (±standard deviation, SD) of trace elements in the collected freshwater samples.

		June 2016	September 2016
Trace elements [μgL^{-1}]	**Li**	0.054±0.011 – 0.529±0.022	0.1120±0.0020 – 0.379±0.017
	Be	0.0020±0.0010 – 0.0110±0.0070	0.0020±0.0010 – 0.0130±0.0060
	Al	0.429±0.021 – 3.456±0.041	1.233±0.053 – 5.92±0.12
	V	0.0300±0.0070 – 0.099±0.013	0.0130±0.0030 – 0.1190±0.0040
	Cr	0.0180±0.0070 – 0.25±0.10	0.0070±0.0010 – 0.176±0.082
	Mn	0.0020±0.0010 – 0.0250±0.0030	0.0120±0.0020 – 0.318±0.014
	Co	0.0040±0.0010 – 0.0380±0.0020	0.0100±0.0010 – 0.0290±0.0050
	Ni	0.073±0.021 – 0.305±0.025	0.124±0.029 – 0.322±0.020
	Cu	0.091±0.011 – 2.33±0.30	0.170±0.019 – 0.819±0.059
	Zn	0.049±0.012 – 2.04±0.57	0.029±0.011 – 0.128±0.017
	Ga	0.0380±0.0070 – 0.183±0.017	0.0790±0.0070 – 0.2720±0.0070
	As	0.136±0.035 – 0.451±0.062	0.108±0.013 – 2.40±0.13
	Rb	0.1900±0.0060 – 0.415±0.016	0.2050±0.0060 – 0.6020±0.0090
	Sr	4.156±0.032 – 33.01±0.20	16.13±0.26 – 43.44±0.78
	Ba	1.855±0.014 – 9.241±0.053	3.384±0.076 – 15.79±0.29
	Tl	0.0110±0.0020 – 0.0160±0.0030	0.0120±0.0030 – 0.095±0.019
	U	0.0100±0.0010 – 0.50±0.22	0.0100±0.0020 – 1.168±0.031

Table 4. Concentrations (±standard deviation, SD) of PAHs, formaldehyde and the sum of phenols in the collected freshwater samples.

		June 2016	September 2016
PAHs [ng L^{-1}]	**Naphthalene (NAP)**	150±23– 611±40	87±10 – 318±22
	Acenaphthylene (ACY)	1.06±0.24 – 47±12	0.43±0.12– 27.3±8.3
	Acenaphthene (ACE)	2.9±1.2 – 57±16	2.1±1.0–111±12
	Fluorene (FL)	3.0±1.1–371±29	6.6±1.9–318±21
	Phenanthrene (PHE)	24.1±7.6 – 30.2±7.6	3.6±1.2 – 368±34
	Anthracene (ANT)	8.9±2.1 – 1871±45	38.0±9.3 – 599±31
	Fluoranthene (FLA)	7.4±1.9 – 985±41	21.9±6.6 – 991±48
	Pyrene (PYR)	3.3±1.2 – 21.3±4.2	2.64±0.89 – 293±20
Phenolic compounds, HCHO [mg L^{-1}]	**∑ Phenols**	0.0120±0.008 – 0.078±0.019	0.031±0.010 – 0.085±0.020
	HCHO	0.160±0.066– 0.53±0.19	0.130±0.054 – 0.29±0.17

Table 5. The list of members of bacterial community possibly involved in the degradation of pollutants. A genus was considered only if it constituted ≥ 0.01% of the community from a single sample.

Bacterial strains	R4-J	R4-S	R8-J	R8-S	R14-J	R14-S	Substrate	References
Achromobacter	0.12%	0.08%	0.12%	0.09%	0.20%	0.07%	PHE	Andreoni et al. 2004
Acidovorax	0.21%	0.30%	0.19%	0.38%	0.19%	0.34%	PHE	Eriksson et al. 2003; Martin et al. 2012
Acinetobacter	0.08%	0.05%	0.04%	0.02%	0.07%	0.10%	NAP, ANT, PHE, ACE, ACY	Ryu et al. 1989; Lal and Khanna 1996; Ghosal et al. 2013
Actinocatenispora	0.17%	0.22%	0.56%	0.21%	0.41%	0.17%	FL	Al-Mueini et al. 2007
Arthrobacter	0.05%	0.03%	0.03%	0.03%	0.08%	0.05%	FL, PHE	Grifoll et al. 1992; Casellas et al. 1997; Seo et al. 2006; Samanta et al. 1999
Bacillus	0.14%	0.16%	0.03%	0.03%	0.09%	0.13%	NAP, PYR	Kumar et al. 2007; Kazunga and Aitken 2000
Burkholderia	0.21%	0.26%	0.07%	0.07%	0.17%	0.19%	NAP, PHE	Balashova et al. 1999; Seo et al. 2007; Laurie and Lloyd-Jones 1999a; 1999b
Cycloclasticus	0.02%	0.02%	0.01%	<0.01%	0.02%	0.02%	NAP, ANT, PHE, FL, PYR	Kasai et al. 2003; Geiselbrecht et al. 1998; Dyksterhouse et al. 1995; Wang et al. 2008; Kappell et al. 2014
Dechloromonas	0.01%	0.02%	0.01%	0.01%	0.02%	0.02%	NAP, ANT, PHE, FL, PYR	Coates et al. 2001a
Desulfosporosinus	0.22%	0.07%	0.01%	0.01%	0.05%	0.04%	Toluene	Sun st al. 2014
Desulfotomaculum	0.16%	0.13%	0.03%	0.02%	0.11%	0.03%	Biphenyl	Selesi and Meckenstock 2009
Desulfovibrio	0.26%	0.15%	0.10%	0.07%	0.22%	0.21%	NAP, ANT, PHE, FL, PYR	Villanueva et al. 2008

Flavobacterium	4.44%	2.75%	4.54%	2.19%	3.36%	1.55%	NAP	Widada et al. 2002; Kappell et al. 2014
Geobacillus	0.05%	0.02%	<0.01%	<0.01%	0.01%	0.01%	NAP	Bubinas et al. 2008
Geobacter	1.74%	0.96%	0.57%	0.16%	0.97%	0.92%	Benzoate	Coates et al. 2001b
Janibacter	0.01%	0.02%	<0.01%	0.01%	0.01%	0.01%	FL, PHE. ANT	Yamazoe et al. 2004
Marinobacter	0.01%	0.01%	<0.01%	0.01%	0.01%	0.02%	NAP, ANT, PHE	Al-Mailem et al. 2013; Kappell et al. 2014
Marinobacterium	0.01%	<0.01%	0.01%	<0.01%	0.02%	0.01%	NAP	Hedlund et al. 2001
Methylobacterium	0.03%	0.03%	0.01%	0.01%	0.02%	0.04%	PHE	Andreoni et al. 2004
Micrococcus	0.01%	<0.01%	0.01%	<0.01%	<0.01%	<0.01%	PHE	Ghosh and Mishra 1983
Moraxella	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	<0.01%	NAP	Tagger et al.. 1990
Mycobacterium	0.09%	0.07%	0.32%	0.21%	0.15%	0.12%	PYR, NAP, PHE, FLA, ANT, FL	Boldrin et al.. 1993; Schneider et al. 1996; Heitkamp et al. 1988; Churchill et al. 2008; Lee et al.. 2007; Van Herwijnen et al. 2003
Mycobacterium vanbaalenii	<0.01%	<0.01%	0.04%	0.02%	0.01%	0.01%	NAP, ANT, PHE, FLA, PYR	Kelley et al. 1990; Moody et al. 2001; Kelley et al. 1993; Kim et al. 2005
Nocardia	0.01%	<0.01%	0.01%	<0.01%	0.01%	0.12%	NAP, ANT, PHE	Zeinali et al. 2008a; 2008b
Nocardioides	0.05%	0.18%	0.01%	0.02%	0.02%	0.07%	PHE	Iwabuchi and Harayama 1997; Iwabuchi and Harayama 1998
Novosphingobium	0.27%	0.62%	0.05%	0.14%	0.34%	0.34%	NAP	Suzuki and Hiraishi 2007
Ochrobactrum	<0.01%	<0.01%	<0.01%	0.02%	0.01%	0.01%	PHE	Ghosal et al. 2010
Paenibacillus	0.07%	0.09%	0.03%	0.01%	0.18%	0.05%	NAP	Daane et al. 2001; 2002
Paracoccus	0.01%	0.01%	0.01%	0.13%	0.01%	0.07%	ANT, PHE, FL	Zhang et al. 2004
Pasteurella	0.07%	0.03%	0.41%	0.22%	0.22%	0.10%	FLA	Sepic and Leskovsek 1999
Polaromonas	5.08%	3.65%	2.40%	1.85%	2.49%	2.08%	NAP	Jeon et al. 2006
Pseudoalteromonas	0.18%	0.12%	0.06%	0.03%	0.12%	0.24%	NAP,. PHE, FL	Hedlund and Staley 2006
Pseudomonas	0.19%	0.17%	0.09%	0.05%	0.25%	0.25%	PHE, NAP, PYR, ACE	Romero et al. 1998; Caldini et al. 1995;

								Weissenfels et al. 1990; Tian et al. 2003; Balashova et al. 1999; Kazunga and Aitken 2000; Prabhu and Phale 2003; Bosch et al. 1999
Ralstonia	0.06%	0.04%	0.03%	0.02%	0.05%	0.07%	NAP	Fuenmayor et al. 1998
Rhizobium	0.02%	0.01%	0.01%	<0.01%	0.01%	0.03%	ACE	Poonthrigpun et al. 2006
Rhodococcus	0.11%	0.19%	0.07%	0.09%	0.08%	0.10%	NAP, FL, ANT, FLA, PYR	Di Gennaro et al. 200; Dean-Ross et al. 2001; Dean-Ross et al. 2002; Walter et al. 1991
Rhodoferax	2.68%	5.60%	1.57%	4.19%	1.56%	2.84%	PHE	Martin et al. 2012
Shewanella	0.06%	0.04%	0.02%	0.02%	0.04%	0.08%	NAP	Hilyard et al. 2008
Sphingobium	0.07%	0.03%	0.01%	<0.01%	0.02%	0.03%	NAP, PHE, ANT, FLA	Cavalca et al. 2007; Chadhain et al. 2007; Roy et al. 2012; Khara 2014; Keum et al. 2006
Sphingomonas	0.74%	0.80%	0.19%	0.44%	0.47%	0.98%	ACE, PHE, ANT, FLA, BaP, PYR	Pinyakong et al. 2004; Wattiau et al. 2001; Van Herwijnen et al.. 2003b; Liu et al. 2004; Rentz et al. 2008; Kazunga and Aitken 2000
Staphylococcus	0.03%	<0.01%	0.04%	0.02%	0.01%	<0.01%	PHE	Mallick et al. 2007

NAP- naphthalene; ANT-anthracene; ACE-Acenaphthene; PHE-phenanthrene; FL-Fluorene; FLA-fluoranthene; PYR-pyrene; BaP-benzo[*a*]pyrene; BaA-benz[*a*]anthracene.