Kosek K., Kozak K., Kozioł K., Jankowska K., Chmiel S., Polkowska Ż., The interaction between bacterial abundance and selected pollutants concentration levels in an arctic catchment (southwest Spitsbergen, Svalbard), SCIENCE OF THE TOTAL ENVIRONMENT, Vol. 622-623 (2018), pp.913-923, DOI: 10.1016/j.scitotenv.2017.11.342

The interaction between bacterial abundance and selected pollutants concentration levels in an arctic catchment (southwest Spitsbergen, Svalbard)

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Abstract: Persistent organic pollutants (POPs) have been a topic of interest in environmental sciences for more than 60 years. POPs in the Arctic have been investigated since the 1970s, when first atmospheric measurements revealed the presence of these pollutants in the polar regions. Major contaminant transport routes to the Arctic include atmospheric and oceanic transport, as well as inflow from rivers and sea ice. The sources of pollutants, such as industry, power generators, vehicle and ship exhausts, introduce the PAHs, phenols, formaldehyde or metals into the Arctic. Transport via sea currents, however, can take several years.The highest concentration levels of total PAHs were observed in two samples from the tributaries in July 2015 and were 1069 ng L^{-1} and 3141 ng L^{-1} and in September 2015, the highest concentrations were observed in samples collected from Revvatnet lake and were 978 ng L^{-1} and 1823 ng L^{-1}. The highest concentrations of trace elements in both months were 41 μg L^{-1} in the sample from the highest tributary (July 2015) and 79 μg L^{-1} in the same sample (September 2015). The purpose of this study was also to determine abundance of bacteria in the Arctic freshwater of different types. Microbes are omnipresent and represent diverse biological communities. In the freshwater ecosystems, microorganisms form the base of the food chain supporting higher trophic levels. Although microbes are generally thought to live in the warm regions of Earth, many of them develop in cold climates. In the Revelva catchment, the biggest number of bacteria were detected at the river estuary in July 2015 and at the sampling point located in the Revvatnet lake in September 2015. Generally, the bacterial abundance indices depended on nutrient levels to a small extent, showing the environment of the Revelva catchment not to be nutrient limited, which is in accordance with its rich biological life also in macroscale.

Keywords: Arctic, contaminants, POPs, bacteria, environmental changes

1. Introduction

The Arctic is no longer considered as a highly pristine environment, although the air, water, soil and sediment concentrations reveal considerably lower levels of contaminants as compared to those found in temperate regions (Kallenborn et al. 2012). Despite this, some characteristic features of the Arctic, e.g. low temperatures, snow precipitation, ice coverage, extended periods of darkness during winter, mean that is has the potential to accumulate certain globally transported contaminants including POPs (Hung et al. 2010).One of the most well-known hypotheses in global atmospheric transport of POPs is the process of global distillation, forcing contaminants out of the warmer regions (including temperate industrial areas) into the polar areas, resulting in a complex POP distribution cycle (Kallenborn et al. 2015). Under ambient temperature conditions, many contaminants have partitioning properties that would allow them to move from one environmental medium to another.

Therefore, these chemicals tend also to vaporize at elevated ambient temperature and condense at lower air temperatures at higher latitudes (Kallenborn et al. 2015). In general, the air masses in the temperate zones strive for a temperature balance with the cold polar air masses. Due to low average annual temperature and the special seasonal daylight conditions in the polar regions, the deposition of persistent pollutants is favoured there (Kallenborn et al. 2012). Persistent organic pollutants (POPs) are toxic substances produced by industries or arising as a result of natural phenomena, and released to the environment. Within the environment, POPs are resistant to degradation and may be accumulated over long periods of time in liquid, solid or gas-phase reservoirs from which they enter food webs (Ma et al. 2016). Persistent anthropogenic pollutants can be transported via the atmosphere, ocean currents and rivers into the Arctic. After entering the polar environment, the chemicals are redistributed within the region by the same transport pathways, in addition to transpolar ice transport and the incorporation into the biological systems through accumulation in the food web. Each step along these transport and redistribution pathways to and within the Arctic is influenced by the current climate change due to its influence on adsorption processes, reactivity and accumulation processes (Kallenborn et al. 2015). The exchange of POPs between the Arctic reservoirs depends critically on physicochemical properties of a pollutant and environmental conditions. With respect to the redistribution of POPs, important intrinsic physicochemical properties including vapour pressure (P_A), Henry's Law constant (H), partition coefficients for octanol-water (K_{OW}), and partition coefficients for octanol-air (K_{OA}), and susceptibility to degradation or transformation (photolysis, hydrolysis, metabolism), provide kinetic controls on how long a chemical may remain in any given compartment. For example, rising temperatures will increase Henry's Law constant, promoting higher partitioning from water into air for POPs (Ma et al. 2016). The presence of every POP in the ambient environment of remote areas, such as the Arctic, shows the efficacy of atmospheric and oceanic transport to these remote regions from the temperate zones, where most of toxic substances have been released (Ma et al. 2016; Mackay and Wania 1995). In addition to the POPs in the Arctic, there are also other chemical compounds including contaminants (e.g. metals, ions), which concentration levels are visible in polar environment. The knowledge of their concentration is a key element in the interpretation of the results for the presence of bacteria. Metals occur naturally in the environment and are present in the soil and rocks in many forms. They can be bound in organic and inorganic molecules or attached to particles in the air. Both, natural and anthropogenic processes have an impact on the changes occurring in the Arctic. Microorganisms depend on some metals as micronutrients but some forms of metals can be toxic for them, even in relatively small amounts (Kozak et al. 2016). Other contaminants that appear in the Arctic are phenols and formaldehyde. They should not be considered less harmful than polycyclic aromatic hydrocarbons or polychlorinated biphenyls. Besides anthropogenic sources, formaldehyde can be also emitted from the snowpack after polar sunrise (Sumner et al. 2002). The low annual average temperatures of the polar regions slow down the microbiological degradation of organic compounds to a minimum. That fact extends the lifetime of chemicals exponentially in the Arctic (Kallenborn et al. 2015).

Climate change is transforming ecosystems on an extraordinary scale, and at an extraordinary pace. As each species responds to its changing environment, its interactions with the physical world and the organisms around it change, too. It starts a cascade of impacts throughout the entire ecosystem. These impacts may include expansion of species into new areas, intermingling of formerly non-overlapping species and even species extinction. Rapid climate change in the Arctic has begun to affect the ecology of animals and plants throughout this polar region with impacts on species ranges, population dynamics and also food web interactions (Grebmeier et al. 2006; Post et al. 2009). Despite this, little attention has been given to the impacts on the microorganisms living in the Arctic. Some of these organisms appear to be in a rapid decline, whereas others are shifting towards new states with implications for food webs and biogeochemical fluxes including pollutants emissions. Global warming leads to dwindling of the cryosphere, the ensemble of ice-containing environments on the Earth. The constant decline in annual sea ice is reducing the growing season for bacterial habitats that live in brine channels between the ice crystals and in the water column at the edge of the observed reduction in benthic respiration rates (Grebmeier et al. 2006). It may be expected that climate change will lead to the disappearance of many types of biota (Vincent 2010). In the Arctic, the number of freshwater reservoirs' is increasing. Melting sea ice combined with freshwater delivery from river inflows is affecting the physical characteristics of the Arctic Ocean by causing reductions in the salinity of the surface mixed layer, which, in turn, results in greater hydrodynamic stability of the water column. These changes have been accompanied by increased bacterial concentrations (Li et al. 2009). Arctic climate change influence also freshwater bacterial ecosystems. Warmer water temperature, erosion and greater evaporation cause some polar lakes and ponds to dry up, which leads to the extinction of many bacterial communities. Furthermore, the bacterial communities inhabiting freshwater ecosystems have the potential to produce globally significant effluxes of greenhouse gases due to bacterial respiration and methanogenesis (Laurion et al. 2010). Finally, emission of POPs to the environment can have an impact on the bacteria living in the Arctic by modifying their local environment (physical aspect) and their nutrient sources and interactions in the food web (biotic aspect) (Boeuf et al. 2014).

The main purpose of the conducted research was to study the interactions between the pollutants and bacterial abundance. In particular, selected xenobiotics, such as PAHs, phenolic compounds and formaldehyde, were determined in an arctic catchment, as were the total number of bacteria, size and biomass. Statistical analysis has been applied to elaborate on their possible interactions.

2. Materials and Methods

2.1. Study area

High latitude ecosystems may be typically thought of as extreme environments containing low amount of contaminants. However, there is increasing evidence, that such a view is not correct. The Svalbard archipelago, due to its specific geographical location and hydrological regimes, may be defined as a place in which pollutants are permanently deposited (Kozak et al. 2013). The Revelva catchment is located in the Wedel-Jarlsberg Land, in southwestern Spitsbergen. The main river (Revelva) is fed both directly by atmospheric precipitation, snow melt water streams and a river originating from the Arie glacier. Revelva drains into the bay of Ariebukta in the south, forming an estuary. In the upper part of the catchment, the main streams originate from the slopes of Eimfjellet (640 masl) and Skålfjellet (635 m asl). The catchment is characterised by an asymmetry, with a predominance of left tributaries, of which the proglacial Ariebekken is the largest. The sampling location has been chosen at the left shore of the lake mainly to determine water input from nearby areas and the glacier. The bed of Revelva is majorly paved with pebbles, with occasional boulders and sandy stretches. The rocks eroded and deposited by the river belong to the Precambrian Hecla-Hoek formation (Birkenmajer 1990; Czerny et al. 1993). A small part of the catchment, at the Revelva springs, is built of Eimfjellet lithological groups. The Revelva catchment has only a small glacierised part, however the past glacial activity has left traces in its topography. The bottom part of the Revelva valley is an elevated marine terrace, with abrasion stacks (Matuła et al. 2007). On the terrace, areas of patterned ground and contemporary storm ridges have been formed. The diversity of the catchment landscape provides an ideal setting for a comprehensive study of pollutant deposition processes in different parts of the abiotic environment (Kozak et al. 2016).

2.2. Sampling

Freshwater samples were manually collected from the Revelva catchment located in the vicinity of the Polish Polar Station, Hornsund. In total, 28 samples were collected in summer 2015 (14 samples both in July and September, repeating the same locations, Figure 1). The sampling location has been chosen in a way to preserve the representativeness of the samples. The morphological and hydrological characteristics of the study area have also been taken into account, as well as the distribution of inflows and sources of pollution. The choice of sampling months depended precisely on the atmospheric conditions in Hornsund. July is considered to be a month of ice melting, and as a result of ablation process, pollutants enter the studied environment. In September, there is an increase in atmospheric precipitation with which further pollutants are delivered.

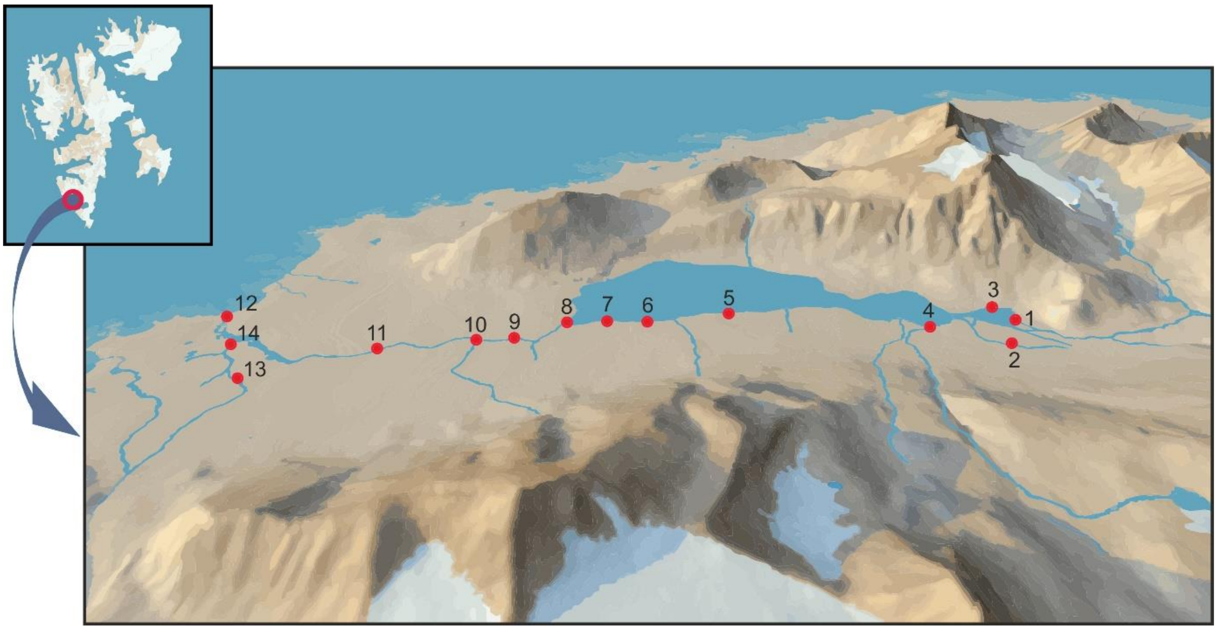


Figure 1. The map of sampled area with the location of sampling points (based on TopoSvalbard portal by Norwegian Polar Institute; toposvalbard.npolar.no).

Freshwater samples were collected from the Revvatnet (lake) and the Revelva (river) at a distance of 1.5 m from the shore with no headspace into air-tight, chemically clean 1L bottles (the purity of the procedure has been verified by daily blank sample collection) using a manual sampling technique. Before sampling campaign, the bottles were precisely cleaned with Milli-Q deionised water (including week-long soaking and removing the water from the sampling containers several times). During watercourse sampling, the neck of the bottle was directed against the stream in order to prevent the inflow of suspended contaminants introduced by the sampling person. The running water was taken from the main stream at depths 20-50 cm below water level. For microbiological analysis, the sub-surface samples were collected with the addition of 2% formaldehyde solution.

2.3. Chemical Analysis

Milli-Q deionised water was used during the determination of the various target analyte groups and to prepare sample container blanks. The concentrations of organic and inorganic compounds were determined in freshwater samples by: Inductively Coupled Plasma Mass Spectrometry, Analyser TOC-VCSH/CSN with 680 °C combustion catalytic oxidation. Sum of phenols and formaldehyde have been determined using Spectrophotometer - Spectroquant Pharo 100 and ions have been determined using ion chromatography coupled with a conductivity detector. PAHs determination was performed with a gas chromatography coupled with a mass spectrometer, fitted with a detector with the electron ionization. Measurements of pH and electrical conductivity (EC) were done using conductivity meter CPC-411 (Elmetron) equipped with an EC60 conductivity sensor; microcomputer pH-meter. Additionally, the temperature of freshwater in the Revelva catchment was measured and ranged from 3 to 5 °C on the surface. Technical specifications for determining selected parameters and analyte contents in the samples, and basic validation parameters of the analytical procedures are summarized in Table 1.

Table 1. Validation parameters and technical specifications used in the applied analytical procedures.

	Determined compounds/parameters	**Measurement range**	**LOD[5]**	**LOQ[5]**	**Measurement Information**
	Electrical conductivity[1]	-	-	-	Electrochemical method: CPC-411 conductometerby Elmetron,conductivity sensor EC60
	pH	-	-	-	Electrochemical method: microcomputer pH-meter by Elmetron, electrode type EPS-1
	TOC[2]	0.150-10.0	0.030	0.100	Total Organic Carbon Analyzer TOC-V$_{CSH/CSN,}$method of catalytic combustion (oxidation) with the application of the NDIR detector
	∑ Phenols[2]	0.002-5.00	0.025	0.075	Spectrophotometer: SpectroquantPharo 100
	Formaldehyde[2]	0.020-8.00	0.020	0.060	
	∑ Anions[2]	0.030-250	0.060	0.180	Ion Chromatograph with conductivity detector (DIONEX ICS-3000)
	∑ Cations[2]	0.030-250	0.010	0.030	
PAHs[3]	**Naphthalene**	1.02-3500	0.034	1.02	Gas Chromatograph 7890A (Agilent Technologies) coupled with a mass spectrometer (5975C inert MSDAgilent Technologies), detector (Agilent
	Acenaphthylene	0.012-1000	0.004	0.012	

	Acenaphthene	0.012-1000	0.004	0.012	Technologies 5975C) with electron ionization
	Fluorene	0.005-1000	0.002	0.005	
	Phenanthrene	0.008-1000	0.003	0.008	
	Anthracene	0.023-1000	0.008	0.023	
	Fluoranthene	0.042-1000	0.014	0.042	
	Pyrene	0.084-1000	0.028	0.084	
Trace elements^4	**Li, Be, Ga, Rb, Tl, U, V,Cr, Mn, Co, Ni, Fe**	0.010-1000	0.010	0.030	Inductively Coupled Plasma Mass Spectrometry (Thermo Scientific XSERIES 2 ICP-MS)
	B, Al, Cu, Zn, As, Ba	0.100-1000	0.100	0.300	
	Sr, P	1.00-1000	1.00	3.00	

^{1}[µS cm^{-1}], ^{2}[mg L^{-1}], ^{3}[ng L^{-1}],^{4}[µg L^{-1}],^{5}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)

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

The analytical procedures applied in the determination of individual components in environmental samples with various matrix compositions have been validated against certified reference materials. The certified reference materials (CRM) were produced and certified in accordance with ISO Guide 34:2009 and ISO/IEC 17025:2005. The data obtained in the research were subjected to strict quality control procedures. Prior to pH measurements, the three-point calibration of the electrode was performed with temperature compensation. For the electrode calibration, MERCK Millipore Certipur®buffer solutions were used: pH 4.01/pH 7.00/pH 9.00 (25°C), which are traceable to SRM from NIST and PTB:; pH 4.01 (phthalate), pH 7.00 (phosphate) and pH 9.00 (borate)., The analysis of metals involved the application of Standard Reference Material NIST 1643e Trace Elementsin Water, and Reference Material Enviro MAT ES-L-2CRM, ES-H-2 CRM SCP SCIENCE. The calibration of the apparatus was based on reference materials by Inorganic ventures ANALITYK: CCS-4, CCS-6, CCS-1, IV-ICPMS-71A. Potassium hydrogen phthalate by Nacalai Tesque (Japan) was used for the calibration of the TOC Analyser. The sensitivity of the applied methods was tested by injecting standard mixtures of the analytes in the measurement range concentration. Linear calibration curves of the peak area against standard concentration showed correlation coefficients (R^2) in the range of0.898–0.999 for all standards. Each sample was analysed in triplicate. During the analysis of the samples, procedural blanks (Milli-Q water with no certified material addition) were prepared for every batch of six samples to check the instrumental background. All the obtained values for PAHs in CRM were within the interval of confidence. Reproducibility and recovery were high (85%–105%) with relative standard deviation (RSD) 4%–10%. Average recoveries of standards in the following validation studies were in range: 70%–85% and of internal standards 80% and 85%, for naphthalene-d8 and benzo[a]anthracene-d12, respectively. All blanks were analysed in the same setup as the samples, using the same reagents. Additionally, formaldehyde and the sum of phenols measurements have been done in accordance with ISO 8466-1 and DIN 38402 A51, respectively.

2.5. Microbiological Analysis

From 1 to 5 ml of the sample has been filtered through a polycarbonate membrane filter with a pore diameter of 0.2 μm and then stained with DAPI (4,6-diamidino-2-phenyl-indol) in a final concentration of 2 μg ml^{-1}. The samples prepared for bacteria detection have been analysed using the epifluorescence microscope Nikon Microscope 80i with NIS-Elements BR 3.0 and MultiScan automated image analysis system. The analysis was carried out using appropriate excitation filters adapted to the used fluorochromes. The total useful microscope magnification was 1200. During analysis, the image analysis system consisting of a snap-in to the microscope Epifani, monochrome high resolution Nikon DS-5Mc-U2 color digital camera. Structure indicators of bacteriocenosis will be estimate based on the results obtained in 20 consecutive fields of view.

2.6. Principal Component Analysis (PCA)

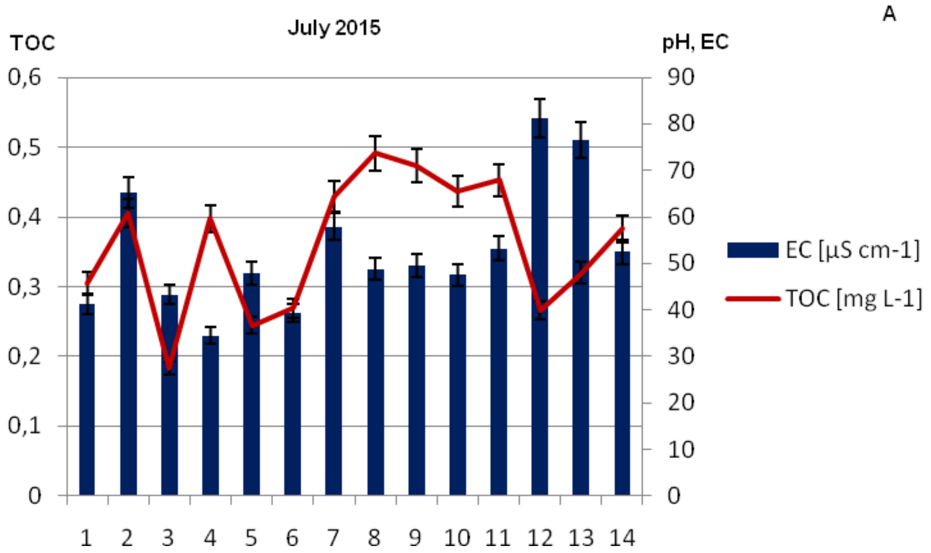
Principal Component Analysis (PCA) is a typical display method that allows to estimate the internal relations in data set. There are various variants of PCA but basically their common feature is that they show linear combination of the original columns in data set responsible for the description of the variables characterising the observation objects. The linear combinations represent a type of abstract measurements (factors, principal components) being better descriptors of the data pattern than the original (chemical or physical) measurements. Usually, the new abstract variables are called latent factors and they differ from the original ones named manifest variables. It is a common finding that just a few of the latent variables account for a large part of the data set variation. Thus, the data structure in a reduced space can be studied (Kozak et al. 2016).

3. Results and Discussion

3.1. Levels of determined contaminants concentrations and other parameters of freshwater samples

Total organic carbon

Total organic carbon (TOC) is the amount of carbon bound in an organic compound and it is the sum of dissolved and suspended organic carbon (DOC+SOC). This constitutes a huge range of compounds with a variety of properties. TOC is released to environment from both natural and anthropogenic sources. All aquatic organisms release TOC through their normal metabolism, excretion and eventual decomposition. Anthropogenic sources include sewage treatment plants, farm slurry and silage runoff. Glacier and alpine lake ecosystems represent two extremely sensitive environments to anthropogenic impacts such as inputs of dust, organic matter and atmospheric pollutants which can be transported over significant distances (Hood et al. 2009; Stubbins et al. 2012; Spencer et al. 2014). Total organic carbon, electrical conductivity and pH have been measured. Carbon is one of the most significant component of any environment, including the polar environment, characterised by an extremely harsh climate. The arctic carbon cycle is an important factor in the global climate system because of the carbon and methane accumulated in the polar regions. If released to the atmosphere, they would increase greenhouse gas concentrations and contribute to climate change (AMAP, 2009). The global climatic changes have significantly affected the cryosphere in many regions of the world, including Svalbard Archipelago (Wang et al. 2013). Glaciers are considered key indicators of climate change due to their rapid reaction to even small climatic shift through melt and mass loss. This could also release pollutants from glaciers to other polar environment reservoirs, as it was mentioned before. Global model simulations indicate that climatic warming is more pronounced at high latitudes in the northern hemisphere and suggest the arctic carbon cycle is extremely sensitive to climate change (Anisimov et al. 2007; Yoshitake et al. 2011). Various studies for the terrestrial Arctic show that land areas are a sink for approximately 300-600 million tonnes of carbon per year. 40-84 million tonnes of carbon is released to the atmosphere from lakes and rivers each year and seawater appears to be a sink for 24-100 · 10^{12} gC yr^{-1}. Carbon is also carried from land to rivers and from rivers to oceans and then it can be emitted to the atmosphere or captured in sediments (McGuire et al. 2009; AMAP 2009). In the collected freshwater samples, TOC ranged from 0.184±0.010 to 0.491±0.010 mg L^{-1} in July and from 0.154±0.010 to 0.903±0.040 mg L^{-1} in September (Fig. 2A,2B).



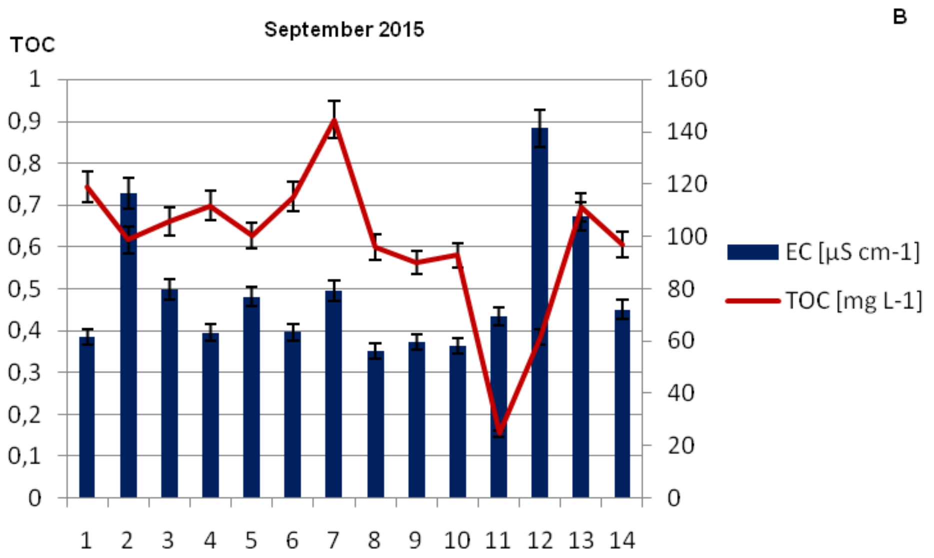


Figure 2A, 2B. Concentration levels of TOC, and the electrical conductivity and pH determined in the collected freshwater samples.

Transport and fate of TOC in aquatic systems are both determined by the local patterns of precipitation, physical and biological interactions, runoff and outflows from glaciers (Hood et al. 2003). Taking into consideration, average results of precipitation collected in July (7.9 mm), August (73.0 mm) and September (171.4 mm), it may be stated that large amount of total organic carbon determined in studied area is delivered from precipitation (http://hornsund.igf.edu.pl/Biuletyny/BIULETYN_38). TOC load in aquatic ecosystems is a mixture of organic material derived from the soil and plants of the surrounding catchment and from within the aquatic ecosystem, derived from algal and bacterial biomass. Relative balance of all TOC sources in aquatic ecosystems may affect the aquatic biota by altering the amount of energy available for heterotrophic growth (Hood et al. 2003).

Besides TOC measurements, electrical conductivity and pH have been checked in freshwater samples collected in 2015. The hydrochemical studies of polar areas were carried out over many years in the surroundings of the Hornsund fjord, also in the catchment of Revelva, and demonstrated high hydrochemical variability. Nevertheless, some of the parameters have been marked at similar ranges, for example, all samples collected in previous years, and also these collected in July and September 2015, were characterised by slightly acidic pH (Fig 2A, 2B) (Ruman et al. 2012; Kozak et al. 2016).

To sum up, TOC plays an important role in many aquatic reservoirs – for example: by providing an energy source for freshwater food webs through bacterial assimilation or by influencing the bioavailability of metals and other toxic chemicals. It can be well seen in collected samples from Revelva catchment. Toxic chemicals (e.g. PAHs) detected in Revvatnet lake and Revelva river have become less harmful to living bacteria. It is also confirmed by the performed PCA analysis and it may be concluded that the presence of TOC in studied waters influence the effect of toxic compounds on bacteria.

Organic compounds

Of the contaminants determined in the collected samples, one group belongs to persistent organic pollutants (POPs): the polycyclic aromatic hydrocarbons (PAHs). Their concentration levels, as well as the sum of phenols and formaldehyde concentrations detected in the water of Revelva catchment, are reported in Table 2.

Table 2. Concentration levels (± standard deviation, SD) of PAHs, sum of phenols and formaldehyde determined in the collected freshwater samples.

		July 2015	**September 2015**
PAHs **[ng L^{-1}]**	**Naphthalene**	108±11 — 518±40	76.1±9.6 — 1823±41
	Acenaphthylene	1.30±0.18 — 52.5±8.7	0.70±0.41 — 17.6±1.6

	Acenaphthene	0.75±0.32 — 44±16	3.80±0.23 — 146±26
	Fluorene	3.8±2.9 — 219±31	5.80±0.70 — 986±29
	Phenanthrene	9.7±7.0 — 29±12	0.70±0.30 — 58.3±8.6
	Anthracene	21±12 — 1450±59	23.7±4.9 — 1342±42
	Fluoranthene	6.6±1.2 — 853±81	6.90±0.91 — 898±24
	Pyrene	2.14±0.21 — 14.3±9.7	2.10±0.21 — 244±21
Phenolic compounds, Formaldehyde [mg L^{-1}]	**∑ Phenols**	0.031±0.010	0.037±0.011 — 0.293±0.041
	Formaldehyde	0.040±0.010 — 0.140±0.036	0.070±0.011 — 0.227±0.042

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are formed during the incomplete burning of coal, biomass, fossil fuels and other industrial activities. They have been found in the Arctic environment, and the long-range atmospheric transport is believed to be one of their primary entry routes (Wang et al. 2013). Alongside the anthropogenic sources of PAHs, they are also emitted to the atmosphere from volcanic eruptions and forest burning. PAHs are an important class of POPs, and many of them are carcinogenic, mutagenic and toxic contaminants. PAHs have been found widely in polar environmental media, such as atmosphere, water, ice, snow, 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 2. The highest concentration levels of PAHs have been detected in the sampling point 13 in July and in the sampling point 8 in September, and these were 1450±59ng L^{-1} and 1342±42ng L^{-1}, respectively.

Such high concentrations of PAHs observed in the sampling point 13 (located at the outflow from the Arie glacier) in July can be easily explained. The Arie glacier is the largest in the Revelva catchment, and hence it may also be a large reservoir of pollutants. In July, ice and snow melting are the main sources of water in the stream heading to the sampling point 13. Furthermore, in this period, the valley bottom becomes marshy and wet, and reindeer herds pass through the area, feeding on moss. The excrement of the reindeers can be an additional source of the PAHs detected in sampling point 13. Figure 3 presents the variable sums of PAHs detected in July and September 2015.

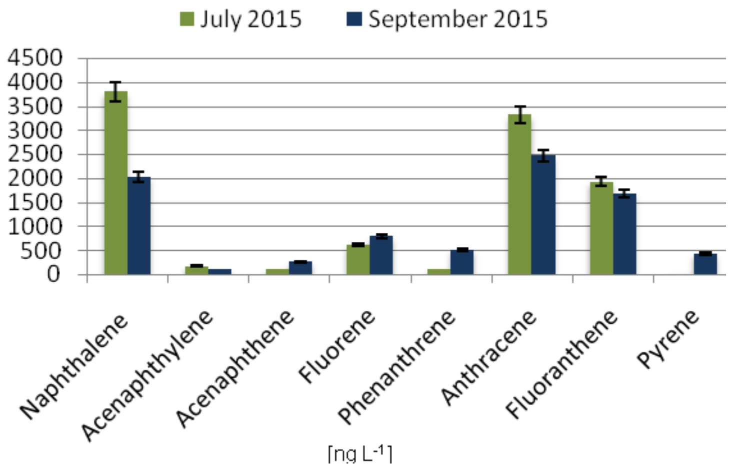


Figure 3. The average concentration levels of the determined PAH congeners in freshwater samples collected in July and September 2015.

Trace elements

Trace elements were also determined in the samples collected in July and September 2015 form the Revelva catchment. The concentrations of the following trace elements were determined in the collected samples: Li, Be, B, Al, P, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ba, Tl, and U (Table 3). The concentration levels of the trace metals detected in freshwater ranged from 12.4 to 41.5 μg L^{-1} in July 2015 and from 36.2 to 80.9 μg L^{-1} in September 2015. Metals concentration CV ranged from 0.5 to 1.5%.

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

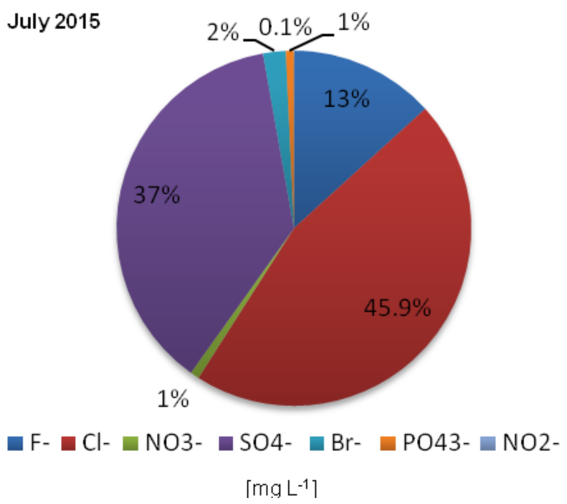
		July 2015	**September 2015**
Trace elements [μg L^{-1}]	**Li**	0.010±0.010 — 0.21±0.30	0.010±0.010 — 0.21±0.19
	Be	<LOD	<LOD
	B	0.16±0.21 — 5.79±0.87	0.14±0.11 — 1.67±0.21
	Al	0.59±0.41 — 3.48±0.23	0.33±0.18 — 9.2±2.8
	P	1.73±0.34 — 10.4±2.9	3.5±2.8 — 29.4±5.2
	V	0.010±0.012 — 0.070±0.019	0.010±0.011 — 0.14±0.27
	Cr	0.010±0.010 — 0.030±0.013	0.010±0.010 — 0.070±0.018
	Mn	0.020±0.011 — 0.36±0.41	0.010±0.011 — 3.88±0.24
	Fe	0.14±0.28 — 1.75±0.19	0.010±0.011 — 2.63±0.91
	Co	0.010±0.012	0.010±0.010 — 0.26±0.24
	Ni	0.010±0.011 — 0.15±0.21	0.010±0.012 — 0.23±0.18
	Cu	0.25±0.33 —0.88±0.56	0.020±0.011 — 1.04±0.18
	Zn	0.23±0.29 — 1.15±0.12	0.060±0.018 — 1.59±0.22
	Ga	0.020±0.012 — 0.090±0.017	0.020±0.011 — 0.30±0.25
	As	0.040±0.018 — 0.65±0.42	0.010±0.010 — 0.97±0.63
	Rb	0.060±0.022 — 0.31±0.32	0.16±0.12 — 0.50±0.38
	Sr	5.70±0.87 — 22.2±4.6	14.1±3.9 — 48.4±5.2

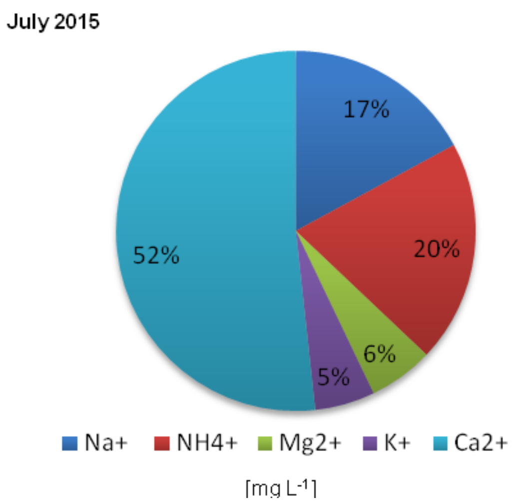
Ba	1.03±0.19 — 5.9±1.2	1.15±0.12 — 11.8±8.3
Tl	0.010±0.011	0.010±0.011 — 0.040±0.016
U	0.010±0.010 — 0.04±0.015	0.010±0.010 — 0.98±0.47

The concentrations detected in two different months varied from each other significantly. The increase of trace element concentrations in September can be well observed. This may be caused both as a result of intensive precipitation in September and consequently greater wet deposition of determined elements and as a result of melting permafrost. Generally, metals detected in polar areas are assumed to be derived mostly from long-range atmospheric transport, geological sources and coal combustions (AMAP 2009). The increase in concentration of trace elements in September 2015 is most evident in the water taken from the point at the shore in the middle of the lake and two points located at the estuary of the river to the fjord. The water flow in the lake, even after heavy rainfall, is smaller than in the river, thus it was possible to observe the increased concentration levels of trace elements there. Moreover, increased concentration of determined compounds at the estuary in September indicates mixing of river and fjord water, as well as mixing of the water from the glacier outflows. Differences in individual trace elements concentration levels can be explained qualitatively in terms of mineral surfaces, complexation, chemical weathering and sorption to solid-phase soil organic matter. The likely influence on these concentrations, besides human activities, are natural processes, especially linked to geological sources. An extra natural source of metals could be the volcanic eruptions in Iceland. The volcanic products may include adsorbed organic and inorganic metal compounds, which would also be deposited after long-range transport in Svalbard (Kozak et al. 2016).

Ions

Figure 5 shows percentage ion concentrations detected in the collected samples. Ion concentration levels give a quantitative information regarding chemical water composition. Lakes and rivers which receive snowmelt and rainwater contain water with low concentration of ions. Because of the source of atmospheric ions, from sea aerosol, chloride and sodium are relatively more abundant than other ions, both in the precipitation and in the surface waters. In the summer of 2015 an average precipitation event contained 48% of cations as Na^+ and 42% of anions as Cl^-. In the collected freshwater samples, Cl^- constitute 45.9% of all detected ions both in July and September 2015, and Na^+ constituted 17% and 34% in July and September 2015, respectively. In addition, rainwater is in equilibrium with atmospheric CO_2 so in effect it becomes a dilute solution of carbonic acid, with an admixture of sea salt and extra sulphate or nitrate lowering its pH further. This was reflected in the volume-weighted summer precipitation pH in the nearby Hornsund of 4.88. Moreover, Revelva catchment is characterised by the rock dominance. Due to it, in the river and lake water samples, there can be observed increased concentrations of phosphates and calcium ions which may be caused as a result of rock weathering; such concentrations were not encountered in precipitation samples in 2015, where the average contribution of Ca^{2+} ions to the cation sum was 6%.





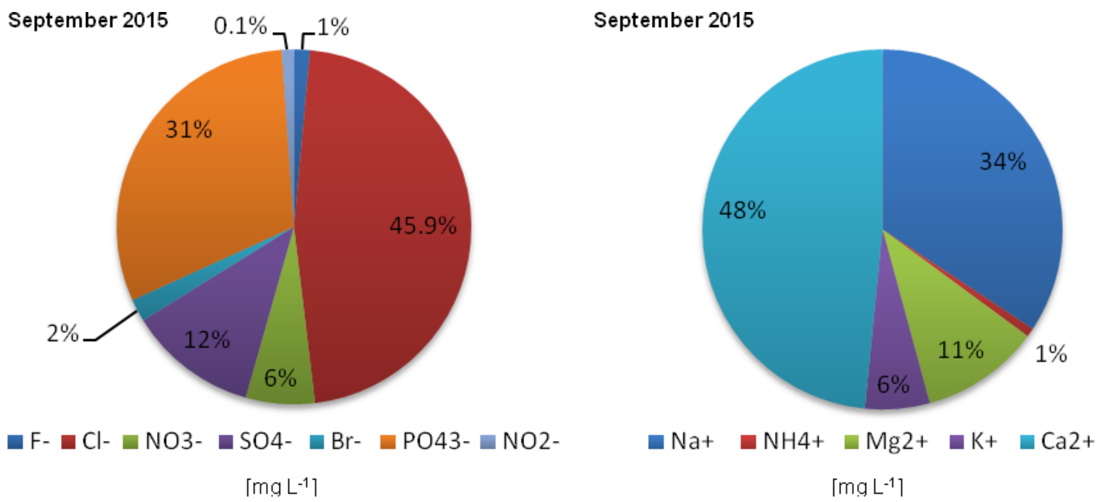


Figure 5. Percentage concentration levels of anions and cations in the collected samples.

3.2. Results of the microbiological analysis

Bacteria are the smallest living organisms but at the same time they are the most abundant ones. Only through field studies the full extent of bacterial diversity can be recognized, as the cultured bacteria are a very limited representation of their environmental populations. Bacteria live at temperatures from less than -20°C to above the water boiling point, and they play a fundamental role in the global biogeochemical cycles. They remineralize organic matter to carbon dioxide, water and inorganic salts. Bacteria are ubiquitous and capable of rapid growth when they have an appropriate quantity of nutrients and conditions favorable for metabolism and cell division. They are also involved in the catalysis and synthesis of organic matter in the aquatic and terrestrial environments. A lot of substances, e.g. cellulose, chitin, pectin, agar, phenols, hydrocarbons may be degraded by bacteria. The rate of decomposition of organic compounds depends on their chemical structure, environmental conditions and complexity (Wu et al. 2010a).

The biomass of bacterial population has been recognised as an important parameter when studying microbial ecology. An estimate of microbial production can be used as a general index of microbial activity and specifically to calculate growth rates. Since many processes scale with it, biomass production can be used to obtain a first-order estimate of rates of several processes mediated by microbes. For example, in case of heterotrophic bacteria, which are the subject of this study, biomass production can be used to estimate the use of dissolved organic carbon if coupled with an estimate of the growth efficiency (Kirchman 2001). In collected freshwater samples, higher bacterial biomass was detected in September 2015, especially in the sampling points 4 and 7. It is strongly linked to average bacterial cell volumes which at these points were also bigger. In the collected samples, the highest values of bacterial biomass were 9.05 μg C dm^{-3} in sampling point 4 and 11.37 μg C dm^{-3} in sampling point 7, both in September 2015. Accordingly, average cell volumes in sampling points 3 and 7 were 0.13 μm^{3}and 0.30 μm^{3}, respectively.

Figure 6 presents the total number of bacteria, bacterial biomass and average cell volume detected in freshwater samples in both months. It may be seen that in September 2015, in the sampling points 3, 4 and 7, bacteria were more abundant than in July 2015. It is caused by greater nutrients bioavailability and probably less exposure to stress factors. Average bacterial cell volume and bacterial biomass were similar in September 2015 in the sampling point 7. Additionally, it can be observed that the average cell volume increases in areas where the number of bacteria is lower both in July and September. This is due to a greater absorption of nutrients by a smaller amount of microorganisms and, consequently, decreasing bacterial propagation. Moreover, it can be clearly seen from the graphs that both in July and September 201, the total bacterial number increases in sampling point 12. Probably, the bacteria enter the estuary along with the marine waters of the Hornsund Fjord.

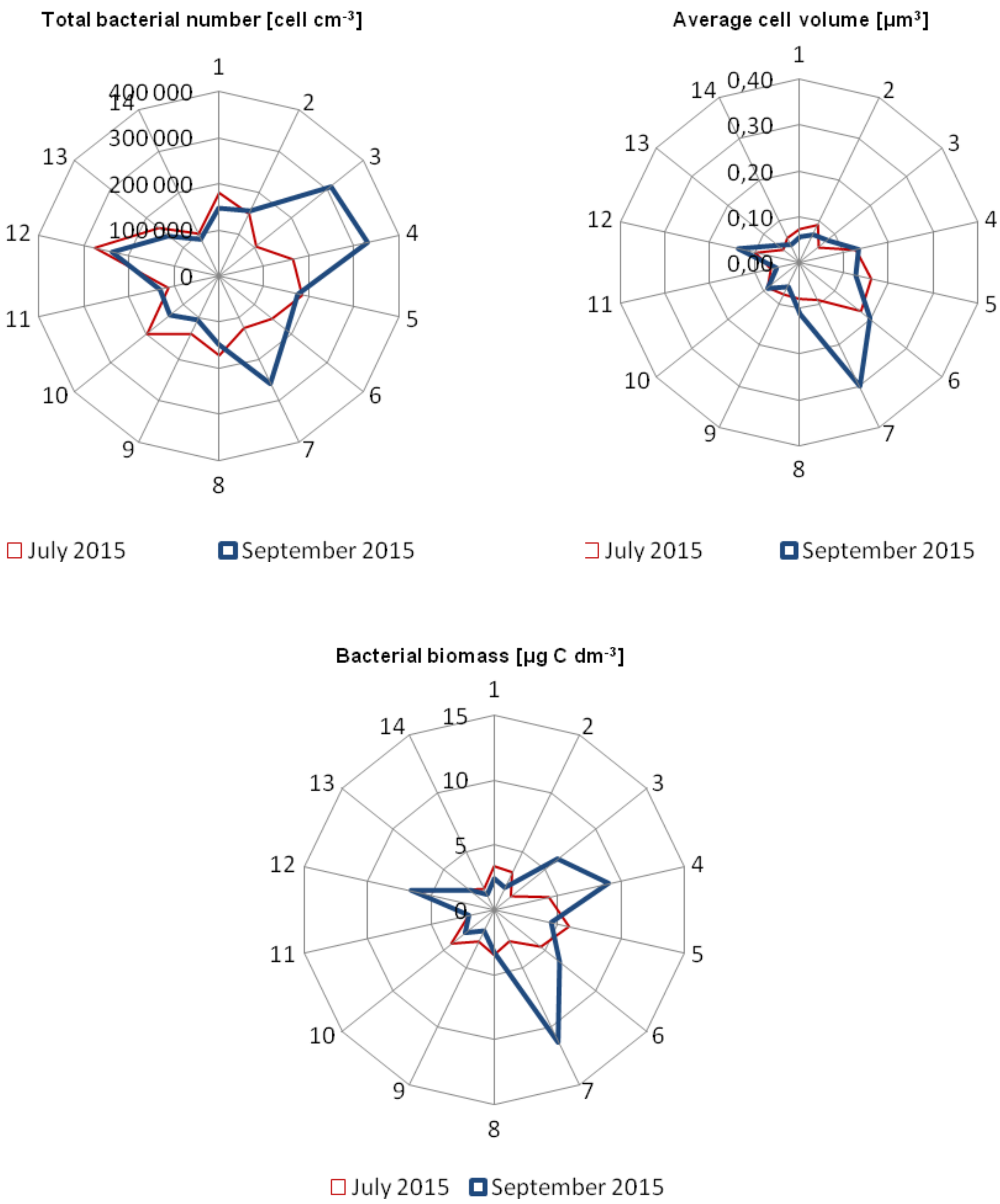


Figure 6. Comparison of bacterial abundance, average bacterial cell volume and bacterial biomass in Revelva catchment in July and September 2015.

3.3. Selected chemical compounds and their influence on bacterial abundance

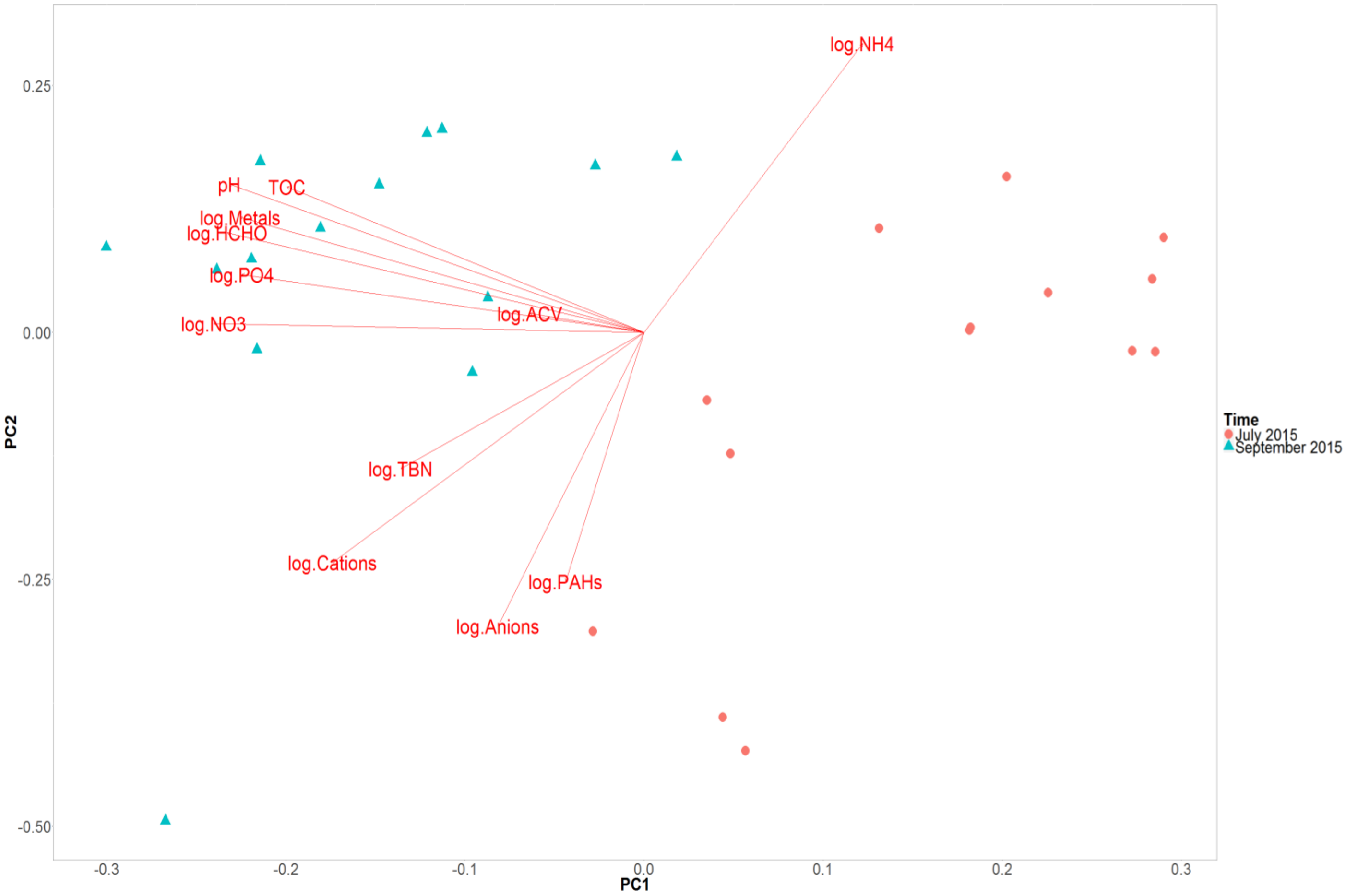
Multiple environmental stressors shape the microbial community locally and regionally. It is of special concern in the polar areas, where the extremely harsh climatic conditions are regarded as main limiting factor. Recently, however, an increasing number of studies (Ntougias et al. 2016; Gillor et al. 2010; Petrone and Richards 2009; Jørgensen et al. 2014;

Stibal et al. 2008)have shown that despite the sparsity of nutrients, exposure to bright sunlight during the summer and prolonged darkness during winter, persistent subfreezing temperatures, such environmental niches harbor relatively abundant life. Nonetheless, the understanding of bacteria community abundance, activity and interactions with a variety of polar stresses is limited. Additionally, these interactions can be fundamentally different than observed elsewhere across the globe (Chu et al., 2010). Therefore it is suspected that bacterial community in the polar regions can be considered a potential degrader of different compounds, even those regarded as anthropogenic-delivered contaminants with limited biodegradation (Chu et al. 2010).

Concentrations of pollutants differ from each other in various aqueous reservoirs (tributaries, lakes and river). Additionally, the interaction of hydrophobic aquatic contaminants with dissolved organic substances and particulate matter may result in the physical partitioning of the compound from the water column, bringing the susceptible substrate into closer association with those bacteria capable of degrading xenobiotics. Such partitioning can also cause a concentration of the contaminant to toxic levels. To this point, solubilization or partitioning of pollutants into dissolved organic phases may stimulate biodegradation through availability of co-metabolizable substrates or inhibition of normal decomposition activity. Many biological compounds (e.g. lipids, nucleic acids, proteins and amino acids) concentrate or increase the solubility of polycyclic aromatic hydrocarbons (Wu et al. 2010a, 2011a). On the other hand, in aquatic environment, there are some bacterial communities incapable of degrading pollutants, and in such areas the concentration levels of contaminants increase significantly. Moreover, an unexpected growth of bacterial communities in the aquatic reservoirs may be also result from bacterial deposition from the atmosphere. Bacteria can be transported thousands of kilometers with atmospheric particles, such as desert dust, which influences atmospheric chemistry, microbial biogeography and the global distribution of clouds and precipitation (Burrows et al. 2009). For example, this trend can be seen in the figure 6. In September 2015, total number of bacteria increases in almost all sampling points, which can be linked to bacteria transport and their deposition with precipitation. Bacteria enter the atmosphere as aerosol particles from almost all surfaces including water, soil and plants (Jones and Harrison 2004). They can be removed from surfaces by gust of wind or mechanical disturbances (e.g. shaking of leaves, surf breaking). Upon entering the air, bacteria may be transported upwards by the air currents and due to their size, remain in the atmosphere for an average period of a few days. They are eventually removed from the atmosphere by dry or wet deposition. The potential for bacteria and also other microorganisms to be transported over long distances has fascinated microbiologists and it has led to the formation of the aerobiology field. The average bacterial residence time in the atmosphere may range from several days to weeks, long enough for cells to travel between continents. Many bacterial defense mechanisms let the organisms withstand the environmental stresses of air transport, such as desiccation, exposure to UV radiation and low pH within cloud water so some bacteria survive this long-range transport to new regions and arrive in a viable state (Burrows et al. 2009). Being aware of this, it may be concluded that not only bacteria that multiply in polar regions are encountered there. Those bacteria which are transported by the air and deposited in high latitudes can develop and proliferate in the Arctic (Burrows et al. 2009).

To explore patterns in the variability of the chemical background and bacterial abundance, we have conducted principal component analysis (PCA) on a set of chosen variables: TBN, ACV, TOC, pH, sum of PAHs, the concentration of formaldehyde (HCHO), the sum of concentration of chosen trace elements, i.e.: Li, Be, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As, Rb, Sr, Ba, Tl, and U (Metals), sums of anions (F^-, Cl^-, Br^- and SO_4^{2-};) and cations (Na^+, K^+, Mg^{2+} and Ca^{2+}), and the concentrations of three nutrients ions: NO_3^-, NH_4^+ and PO_4^{3-}. BB was not included as a variable derived from TBV and ACV, and sum of phenols was omitted to due to overrepresentation of values below LOD. The other <LOD values were replaced by 0.5 LOD. All variables except TOC and pH were log-transformed to reflect the nature of their distribution.

Based on the eigenvalue >1 criterion, we have distinguished three potentially significant principal components, collectively explaining 69.4 % of the variance. In the coordinate system described by the two first principal components, there was a clear division between samples collected in the early and late summer (Figure 8). The variables indicating chemical threats and nutrients were strongly correlated, and much stronger represented in the September samples. This could reflect their biological origin, as is possible in the Arctic tundra ecosystem with a transport vector for both nutrients and pollution represented by the birds feeding in the marine environment (Blais et al., 2005). In this view, the concentrations of inorganic ions and PAHs cluster together, which may indicate a separate source (most likely atmospheric deposition). The ammonium ion was the least correlated with the other viariables (and it may indicate its spreading in the gaseous form of ammonia from the bird colonies in the vicinity, or microbial production).


log.NH4
pH
TOC
log.Metals
log.HCHO
log.PO4
log.ACV
log.NO3
log.TBN
log.Cations
log.PAHs
log.Anions
0.25
0.00
-0.25
-0.50
PC2
-0.3
-0.2
-0.1
0.0
0.1
0.2
0.3
PC1
Time
● July 2015
▲ September 2015

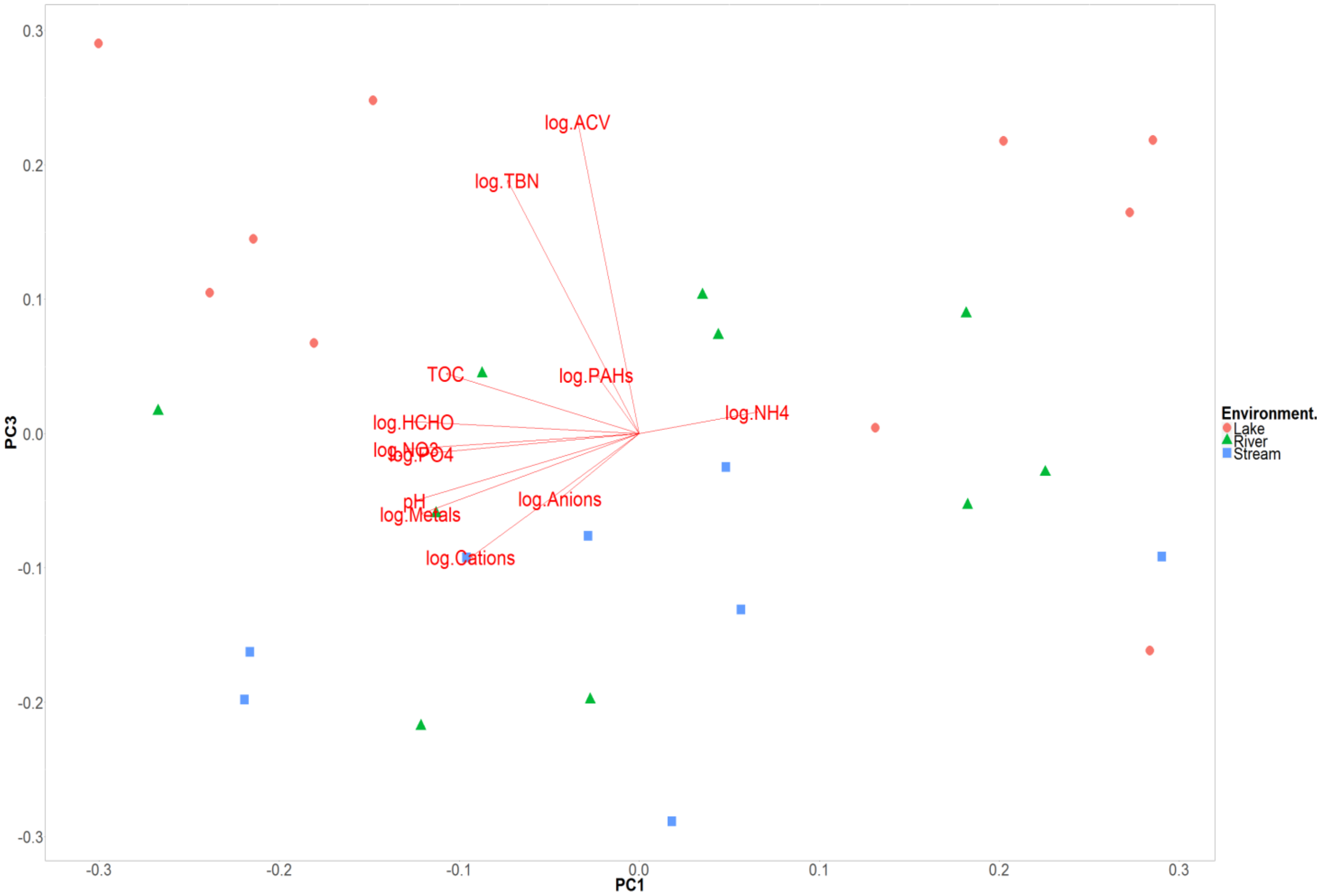


Figure 8. Variables as vectors in the space defined by three principal components described in text (from top to bottom: F1/F2 and F1/F3); points are samples (representing 14 different sites and two dates).

The bacterial abundance indices depended on nutrient levels to a small extent, showing the environment of the Revelva catchment not to be nutrient limited, which is in accordance with its rich biological life also in macroscale. These indices were equally uncorrelated with the chemical threats (pollutant concentrations), which indicates the microorganisms of the Revelva catchment cope well with the experienced levels of pollution. An exception is perhaps the concentration of PAHs, positively correlated with bacterial indices, which shows they may even thrive in the environment polluted by PAHs (which is consistent with the discoveries of PAH-decomposing bacteria on Arctic glaciers (Hauptmann et al., 2017). In the division of sites according to hydrological environment type, the lake waters were most abundant in bacteria, while streams expressed more variability in the scope of inorganic chemical composition (trace elements and ions).

4. Summary

Among polar regions, the Svalbard archipelago is distinguished by its location as the gateway to the Arctic, as well as by its environmental conditions, which make it a pollution reservoir (Ruman et al. 2012). Relatively close location of Svalbard archipelago to Europe makes this sensitive region particularly exposed to the influence of pollutants, both emitted from industrial European areas (located e.g. in the European part of Russia, Great Britain, or Norway), and resulting from natural phenomena (such as volcanic eruptions, forest fires) (Kozak et al. 2016). Additionally, the landscape of Svalbard with its prevalence of mountains, favours the accumulation of pollutants transported by air masses from Europe and Asia. As a result of the processes of wet and dry deposition, contaminants are delivered to the Arctic ecosystems (Laing et al. 2014; Wojtuń et al. 2013). The Revelva catchment, at the Hornsund fjord, has been chosen for a comprehensive study on the pollutants deposition. Revelva catchment can be specified as an ideal area to study deposition of the contaminants introduced to the Arctic environment (Kozak et al. 2016).

The highest concentration levels of ΣPAHs were observed in the sample13 of July 2015and in sample 8 of September 2015,amounting to 3141.56 ng $L^{-1}$and 1823.42 ng L^{-1}, respectively. The highest concentrations of the sum of metals in both moths were found in sample 2, reaching 41.54 μg L^{-1} in July and 79.25 μg L^{-1}in September. The concentration levels of the sum of phenols and formaldehyde were two times higher in September than in July. It result from more frequent precipitation in September and the wet deposition of these pollutants.

Contaminants are deposited into the areas where bacterial life develops. The relationship between them is extremely difficult to study due to multiple processes occurring in the environment and rapidly changing environmental conditions. It should be emphasised that bacterial communities are widely different and divergent from each other in various aspects. Despite it, many of them are characterised by the ability to decompose pollutants. Adsorption by bacteria (often called biosorption) is a mechanism that may be used to remove pollutants from aqueous reservoirs. Selected features of bacteria and some special abilities such as adhesion and flocculation enable the bacterial communities to absorb some heavy metals and toxic materials from the water (Aksu 2005; Sheng et al. 2010).Other mechanisms of pollutant removal by bacteria are: biodegradation, complexation, and ion exchange. Biodegradation is chemical disbanding of organic materials by bacterial communities or other biological agents and involves a complex series of biochemical reactions. It usually varies with the involved bacteria (Wu et al.2010a, 2011a). Complexation plays a significant role in removing heavy metals by bacterial communities. Many functional groups in the extracellular polymeric substances, such as phosphoric, carboxyl, phenolic, hydroxyl and sulfhydryl groups are able to complex with heavy metals and it is rightly described in many previous studies (e.g. Tsuruta 2004; Kao et al. 2006, 2008; Joo et al. 2010). The ion exchange mechanism is the main mode of interaction between some divalent cations and EPS (extracellular polymeric substance). It has been reported that the binding between the EPS and the cations such as Ca^{2+} and Mg^{2+} is one of the main intermolecular interactions supporting bacterial communities' structures. During the removal of metals by bacteria, Ca^{2+} and Mg^{2+} are released into the water, indicating that ion exchange is involved (Wu et al. 2010a, 2011a). On the other hand, in harsh polar conditions, bacterial degradation processes can be inhibited, for example due to the lack of bioavailable nutrients or environmental stresses, hence the interactions between pollutants and bacteria abundance are fuzzy.

Acknowledgments: The authors would like to thank the staff of the Polish Polar Station Hornsund for the opportunity to carry out sampling and for their assistance with this work. Additionally, The National Science Centre of Poland is thanked for the research grant no. 2017/25/N/NZ9/01506. This work was partially supported within statutory activities no. 3841/E-41/S/2017 of the Ministry of Science and Higher Education of Poland.

Conflict of Interests: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analysis, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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