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	11	levels in an arctic catchment (southwest Spitsbergen, Svalbard)
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	22	
	23	Abstract: Persistent organic pollutants (POPs) have been a topic of interest in environmental
	24	sciences for more than 60 years. POPs in the Arctic have been investigated since the 1970s,
zy.pl	25	when first atmospheric measurements revealed the presence of these pollutants in the polar
stwied	26	regions. Major contaminant transport routes to the Arctic include atmospheric and oceanic
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en investigated since the 1970s, of these pollutants in the polar clude atmospheric and oceanic transport, as well as inflow from rivers and sea ice. The sources of pollutants, such as 27 industry, power generators, vehicle and ship exhausts, introduce the PAHs, phenols, 28 formaldehyde or metals into the Arctic. Transport via sea currents, however, can take several 29 years. The highest concentration levels of total PAHs were observed in two samples from the 30 tributaries in July 2015 and were 1069 ng L<sup>-1</sup> and 3141 ng L<sup>-1</sup> and in September 2015, the 31 highest concentrations were observed in samples collected from Revvatnet lake and were 978 32 ng L<sup>-1</sup> and 1823 ng L<sup>-1</sup>. The highest concentrations of trace elements in both months were 41 33

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# The interaction between bacterial abundance and selected pollutants concentration 10 oard)

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 $\mu$ g L<sup>-1</sup> in the sample from the highest tributary (July 2015) and 79  $\mu$ g L<sup>-1</sup> in the same sample 34 35 (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 36 biological communities. In the freshwater ecosystems, microorganisms form the base of the 37 food chain supporting higher trophic levels. Although microbes are generally thought to live 38 in the warm regions of Earth, many of them develop in cold climates. In the Revelva 39 40 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 41 bacterial abundance indices depended on nutrient levels to a small extent, showing the 42 environment of the Revelva catchment not to be nutrient limited, which is in accordance with 43 its rich biological life also in macroscale. 44

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

#### 46 **1. Introduction**

47 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 48 compared to those found in temperate regions (Kallenborn et al. 2012). Despite this, some 49 characteristic features of the Arctic, e.g. low temperatures, snow precipitation, ice coverage, 50 extended periods of darkness during winter, mean that is has the potential to accumulate 51 certain globally transported contaminants including POPs (Hung et al. 2010). One of the most 52 well-known hypotheses in global atmospheric transport of POPs is the process of global 53 54 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. 55 56 2015). Under ambient temperature conditions, many contaminants have partitioning 57 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 58 59 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 60 masses. Due to low average annual temperature and the special seasonal daylight conditions 61 in the polar regions, the deposition of persistent pollutants is favoured there (Kallenborn et al. 62 2012). Persistent organic pollutants (POPs) are toxic substances produced by industries or 63 arising as a result of natural phenomena, and released to the environment. Within the 64 environment, POPs are resistant to degradation and may be accumulated over long periods of 65 time in liquid, solid or gas-phase reservoirs from which they enter food webs (Ma et al. 2016). 66 67 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 68 within the region by the same transport pathways, in addition to transpolar ice transport and 69 70 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 71 72 current climate change due to its influence on adsorption processes, reactivity and 73 accumulation processes (Kallenborn et al. 2015). The exchange of POPs between the Arctic reservoirs depends critically on physicochemical properties of a pollutant and environmental 74 75 conditions. With respect to the redistribution of POPs, important intrinsic physicochemical properties including vapour pressure (P<sub>A</sub>), Henry's Law constant (H), partition coefficients for 76 octanol-water (K<sub>OW</sub>), and partition coefficients for octanol-air (K<sub>OA</sub>), and susceptibility to 77 degradation or transformation (photolysis, hydrolysis, metabolism), provide kinetic controls 78 on how long a chemical may remain in any given compartment. For example, rising 79 temperatures will increase Henry's Law constant, promoting higher partitioning from water 80 into air for POPs (Ma et al. 2016). The presence of every POP in the ambient environment of 81 82 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 83 84 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 85 concentration levels are visible in polar environment. The knowledge of their concentration is 86 a key element in the interpretation of the results for the presence of bacteria. Metals occur 87 naturally in the environment and are present in the soil and rocks in many forms. They can be 88 89 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. 90 Microorganisms depend on some metals as micronutrients but some forms of metals can be 91 92 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 93 harmful than polycyclic aromatic hydrocarbons or polychlorinated biphenyls. Besides 94 95 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 96 97 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). 98

Climate change is transforming ecosystems on an extraordinary scale, and at an extraordinary 99 100 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 101 entire ecosystem. These impacts may include expansion of species into new areas, 102 103 intermingling of formerly non-overlapping species and even species extinction. Rapid climate 104 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 105 interactions (Grebmeier et al. 2006; Post et al. 2009). Despite this, little attention has been 106 107 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 108 109 implications for food webs and biogeochemical fluxes including pollutants emissions. Global warming leads to dwindling of the cryosphere, the ensemble of ice-containing environments 110 on the Earth. The constant decline in annual sea ice is reducing the growing season for 111 bacterial habitats that live in brine channels between the ice crystals and in the water column 112 at the edge of the observed reduction in benthic respiration rates (Grebmeier et al. 2006). It 113 114 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 115 ice combined with freshwater delivery from river inflows is affecting the physical 116 117 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 118 119 changes have been accompanied by increased bacterial concentrations (Li et al. 2009). Arctic 120 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 121 the extinction of many bacterial communities. Furthermore, the bacterial communities 122 123 inhabiting freshwater ecosystems have the potential to produce globally significant effluxes of greenhouse gases due to bacterial respiration and methanogenesis (Laurion et al. 2010). 124 125 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 126 interactions in the food web (biotic aspect) (Boeuf et al. 2014). 127

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.

#### 133 2. Materials and Methods

## 134 2.1. Study area

High latitude ecosystems may be typically thought of as extreme environments containing 135 136 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 137 regimes, may be defined as a place in which pollutants are permanently deposited (Kozak et 138 al. 2013). The Revelva catchment is located in the Wedel-Jarlsberg Land, in southwestern 139 Spitsbergen. The main river (Revelva) is fed both directly by atmospheric precipitation, snow 140 141 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 142 streams originate from the slopes of Eimfjellet (640 masl) and Skålfjellet (635 m asl). The 143 144 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 145 shore of the lake mainly to determine water input from nearby areas and the glacier. The bed 146 of Revelva is majorly paved with pebbles, with occasional boulders and sandy stretches. The 147 rocks eroded and deposited by the river belong to the Precambrian Hecla-Hoek formation 148 149 (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 150 151 part, however the past glacial activity has left traces in its topography. The bottom part of the 152 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 153 diversity of the catchment landscape provides an ideal setting for a comprehensive study of 154 155 pollutant deposition processes in different parts of the abiotic environment (Kozak et al. 156 2016).

157 2.2. Sampling

Freshwater samples were manually collected from the Revelva catchment located in the 158 vicinity of the Polish Polar Station, Hornsund. In total, 28 samples were collected in summer 159 2015 (14 samples both in July and September, repeating the same locations, Figure 1). The 160 sampling location has been chosen in a way to preserve the representativeness of the samples. 161 The morphological and hydrological characteristics of the study area have also been taken 162 into account, as well as the distribution of inflows and sources of pollution. The choice of 163 sampling months depended precisely on the atmospheric conditions in Hornsund. July is 164 considered to be a month of ice melting, and as a result of ablation process, pollutants enter 165 the studied environment. In September, there is an increase in atmospheric precipitation with 166 which further pollutants are delivered. 167

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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 172 173 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 174 manual sampling technique. Before sampling campaign, the bottles were precisely cleaned 175 with Milli-Q deionised water (including week-long soaking and removing the water from the 176 sampling containers several times). During watercourse sampling, the neck of the bottle was 177 178 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 179 depths 20-50 cm below water level. For microbiological analysis, the sub-surface samples 180 181 were collected with the addition of 2% formaldehyde solution.

182 2.3. Chemical Analysis

183 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 184 compounds were determined in freshwater samples by: Inductively Coupled Plasma Mass 185 Spectrometry, Analyser TOC-VCSH/CSN with 680 °C combustion catalytic oxidation. Sum 186 of phenols and formaldehyde have been determined using Spectrophotometer - Spectroquant 187 188 Pharo 100 and ions have been determined using ion chromatography coupled with a conductivity detector. PAHs determination was performed with a gas chromatography 189 coupled with a mass spectrometer, fitted with a detector with the electron ionization. 190 191 Measurements of pH and electrical conductivity (EC) were done using conductivity meter 192 CPC-411 (Elmetron) equipped with an EC60 conductivity sensor; microcomputer pH-meter. Additionally, the temperature of freshwater in the Revelva catchment was measured and 193 194 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 195 196 analytical procedures are summarized in Table 1.

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

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

	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	
	Li, Be, Ga, Rb, Tl,	0.010-1000	0.010	0.030	Inductively Coupled Plasma Mass Spectrometry
4	U, V,Cr, Mn, Co,				(Thermo Scientific XSERIES 2 ICP-MS)
ce elements	Ni, Fe				
	B, Al, Cu, Zn, As,	0.100-1000	0.100	0.300	
Tra	Ba				
	Sr, P	1.00-1000	1.00	3.00	

 $^{1}$ [µS cm<sup>-1</sup>],  $^{2}$ [mg L<sup>-1</sup>],  $^{3}$ [ng L<sup>-1</sup>],  $^{4}$ [µg L<sup>-1</sup>],  $^{5}$ 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)

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The analytical procedures applied in the determination of individual components in 201 202 environmental samples with various matrix compositions have been validated against certified reference materials. The certified reference materials (CRM) were produced and certified in 203 accordance with ISO Guide 34:2009 and ISO/IEC 17025:2005. The data obtained in the 204 205 research were subjected to strict quality control procedures. Prior to pH measurements, the 206 three-point calibration of the electrode was performed with temperature compensation. For the electrode calibration, MERCK Millipore Certipur®buffer solutions were used: pH 207 4.01/pH 7.00/pH 9.00 (25°C), which are traceable to SRM from NIST and PTB:; pH 4.01 208 (phthalate), pH 7.00 (phosphate) and pH 9.00 (borate)., The analysis of metals involved the 209 210 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 211 of the apparatus was based on reference materials by Inorganic ventures ANALITYK: CCS-4, 212 213 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 214 tested by injecting standard mixtures of the analytes in the measurement range concentration. 215 Linear calibration curves of the peak area against standard concentration showed correlation 216 coefficients  $(R^2)$  in the range of 0.898–0.999 for all standards. Each sample was analysed in 217 triplicate. During the analysis of the samples, procedural blanks (Milli-Q water with no 218 certified material addition) were prepared for every batch of six samples to check the 219 220 instrumental background. All the obtained values for PAHs in CRM were within the interval 221 of confidence. Reproducibility and recovery were high (85%-105%) with relative standard 222 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 223 224 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.

228 2.5. Microbiological Analysis

229 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 230 final concentration of 2 µg ml<sup>-1</sup>. The samples prepared for bacteria detection have been 231 analysed using the epifluorescence microscope Nikon Microscope 80i with NIS-Elements BR 232 233 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 234 magnification was 1200. During analysis, the image analysis system consisting of a snap-in to 235 236 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 237 in 20 consecutive fields of view. 238

## 239 2.6. Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a typical display method that allows to estimate the 240 internal relations in data set. There are various variants of PCA but basically their common 241 feature is that they show linear combination of the original columns in data set responsible for 242 243 the description of the variables characterising the observation objects. The linear combinations represent a type of abstract measurements (factors, principal components) being 244 better descriptors of the data pattern than the original (chemical or physical) measurements. 245 246 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 247

account for a large part of the data set variation. Thus, the data structure in a reduced spacecan be studied (Kozak et al. 2016).

## 250 3. Results and Discussion

3.1. Levels of determined contaminants concentrations and other parameters of freshwatersamples

### 253 Total organic carbon

Total organic carbon (TOC) is the amount of carbon bound in an organic compound and it is 254 255 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 256 natural and anthropogenic sources. All aquatic organisms release TOC through their normal 257 258 metabolism, excretion and eventual decomposition. Anthropogenic sources include sewage 259 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, 260 261 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 262 conductivity and pH have been measured. Carbon is one of the most significant component of 263 any environment, including the polar environment, characterised by an extremely harsh 264 265 climate. The arctic carbon cycle is an important factor in the global climate system because of 266 the carbon and methane accumulated in the polar regions. If released to the atmosphere, they 267 would increase greenhouse gas concentrations and contribute to climate change (AMAP, 2009). The global climatic changes have significantly affected the cryosphere in many regions 268 269 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 270 271 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 272 climatic warming is more pronounced at high latitudes in the northern hemisphere and suggest 273 the arctic carbon cycle is extremely sensitive to climate change (Anisimov et al. 2007; 274 Yoshitake et al. 2011). Various studies for the terrestrial Arctic show that land areas are a sink 275 for approximately 300-600 million tonnes of carbon per year. 40-84 million tonnes of carbon 276 is released to the atmosphere from lakes and rivers each year and seawater appears to be a 277 sink for  $24-100 \cdot 10^{12}$  gC yr<sup>-1</sup>. Carbon is also carried from land to rivers and from rivers to 278 279 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 280  $0.491\pm0.010 \text{ mg L}^{-1}$  in July and from  $0.154\pm0.010$  to  $0.903\pm0.040 \text{ mg L}^{-1}$  in September (Fig. 281 2A,2B). 282



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

289 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 290 al. 2003). Taking into consideration, average results of precipitation collected in July (7.9 291 mm), August (73.0 mm) and September (171.4 mm), it may be stated that large amount of 292 293 total organic carbon determined in studied area is delivered from precipitation 294 (http://hornsund.igf.edu.pl/Biuletyny/BIULETYN\_38). TOC load in aquatic ecosystems is a 295 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 296 297 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). 298

Besides TOC measurements, electrical conductivity and pH have been checked in freshwatersamples 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.

#### 313 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	Naphthalene	108±11 — 518±40	76.1±9.6 — 1823±41
[ng L <sup>-1</sup> ]	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	$\sum$ Phenols	0.031±0.010	0.037±0.011 0.293±0.041
compounds, Formaldehyde	Formaldehyde	0.040±0.010 — 0.140±0.036	0.070±0.011 0.227±0.042
[mg L <sup>-1</sup> ]			

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Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are 321 322 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 323 transport is believed to be one of their primary entry routes (Wang et al. 2013). Alongside the 324 325 anthropogenic sources of PAHs, they are also emitted to the atmosphere from volcanic 326 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 327 environmental media, such as atmosphere, water, ice, snow, sediments and biota (Polkowska 328 et al. 2011; Kozak et al. 2017). They can be deposited and accumulated in ice for a long 329 330 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 331 concentration levels of PAHs have been detected in the sampling point 13 in July and in the 332

sampling point 8 in September, and these were 1450±59ng L<sup>-1</sup> and 1342±42ng L<sup>-1</sup>,
respectively.

Such high concentrations of PAHs observed in the sampling point 13 (located at the outflow 335 from the Arie glacier) in July can be easily explained. The Arie glacier is the largest in the 336 Revelva catchment, and hence it may also be a large reservoir of pollutants. In July, ice and 337 snow melting are the main sources of water in the stream heading to the sampling point 13. 338 Furthermore, in this period, the valley bottom becomes marshy and wet, and reindeer herds 339 pass through the area, feeding on moss. The excrement of the reindeers can be an additional 340 source of the PAHs detected in sampling point 13. Figure 3 presents the variable sums of 341 PAHs detected in July and September 2015. 342



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

346 *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  $\mu$ g L<sup>-1</sup> in July 2015 and from 36.2 to 80.9  $\mu$ g L<sup>-1</sup> 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.

		<b>July 2015</b>	September 2015
Trace elements	Li	0.010±0.010 - 0.21±0.30	0.010±0.010 0.21±0.19
[µg L <sup>-1</sup> ]	Be	<lod< th=""><th><lod< th=""></lod<></th></lod<>	<lod< th=""></lod<>
	В	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
	Р	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	$0.010 \pm 0.011 - 0.14 \pm 0.27$
	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
	Со	0.010±0.012	$0.010 \pm 0.010 - 0.26 \pm 0.24$
	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	$0.020 \pm 0.011 - 1.04 \pm 0.18$
	Zn	0.23±0.29 — 1.15±0.12	0.060±0.018 — 1.59±0.22
	Ga	$0.020 \pm 0.012 - 0.090 \pm 0.017$	0.020±0.011 0.30±0.25
	As	$0.040\pm0.018 - 0.65\pm0.42$	$0.010 \pm 0.010 - 0.97 \pm 0.63$
	Rb	$0.060 \pm 0.022 - 0.31 \pm 0.32$	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	$0.010 \pm 0.010 - 0.04 \pm 0.015$	$0.010 \pm 0.010 - 0.98 \pm 0.47$

355

The concentrations detected in two different months varied from each other significantly. The 356 increase of trace element concentrations in September can be well observed. This may be 357 caused both as a result of intensive precipitation in September and consequently greater wet 358 deposition of determined elements and as a result of melting permafrost. Generally, metals 359 360 detected in polar areas are assumed to be derived mostly from long-range atmospheric 361 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 362 363 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, 364 thus it was possible to observe the increased concentration levels of trace elements there. 365 Moreover, increased concentration of determined compounds at the estuary in September 366 367 indicates mixing of river and fjord water, as well as mixing of the water from the glacier 368 outflows. Differences in individual trace elements concentration levels can be explained qualitatively in terms of mineral surfaces, complexation, chemical weathering and sorption to 369 solid-phase soil organic matter. The likely influence on these concentrations, besides human 370 371 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 372 include adsorbed organic and inorganic metal compounds, which would also be deposited 373 after long-range transport in Svalbard (Kozak et al. 2016). 374

375 *Ions* 

Figure 5 shows percentage ion concentrations detected in the collected samples. Ion 376 concentration levels give a quantitative information regarding chemical water composition. 377 Lakes and rivers which receive snowmelt and rainwater contain water with low concentration 378 of ions. Because of the source of atmospheric ions, from sea aerosol, chloride and sodium are 379 relatively more abundant than other ions, both in the precipitation and in the surface waters. In 380 the summer of 2015 an average precipitation event contained 48% of cations as  $Na^+$  and 42% 381 382 of anions as Cl<sup>-</sup>. In the collected freshwater samples, Cl<sup>-</sup> constitute 45.9% of all detected ions both in July and September 2015, and Na<sup>+</sup> constituted 17% and 34% in July and September 383 2015, respectively. In addition, rainwater is in equilibrium with atmospheric  $CO_2$  so in effect 384 it becomes a dilute solution of carbonic acid, with an admixture of sea salt and extra sulphate 385 or nitrate lowering its pH further. This was reflected in the volume-weighted summer 386 precipitation pH in the nearby Hornsund of 4.88. Moreover, Revelva catchment is 387 388 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 389 a result of rock weathering; such concentrations were not encountered in precipitation 390 samples in 2015, where the average contribution of  $Ca^{2+}$  ions to the cation sum was 6%. 391







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

# 395 3.2. Results of the microbiological analysis

Bacteria are the smallest living organisms but at the same time they are the most abundant 396 397 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. 398 Bacteria live at temperatures from less than -20°C to above the water boiling point, and they 399 play a fundamental role in the global biogeochemical cycles. They remineralize organic 400 matter to carbon dioxide, water and inorganic salts. Bacteria are ubiquitous and capable of 401 rapid growth when they have an appropriate quantity of nutrients and conditions favorable for 402 403 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, 404 405 pectin, agar, phenols, hydrocarbons may be degraded by bacteria. The rate of decomposition of organic compounds depends on their chemical structure, environmental conditions and 406 complexity (Wu et al. 2010a). 407

408 The biomass of bacterial population has been recognised as an important parameter when 409 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 410 411 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 412 413 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 414 collected freshwater samples, higher bacterial biomass was detected in September 2015, 415 especially in the sampling points 4 and 7. It is strongly linked to average bacterial cell 416 volumes which at these points were also bigger. In the collected samples, the highest values of 417 bacterial biomass were 9.05 µg C dm<sup>-3</sup> in sampling point 4 and 11.37 µg C dm<sup>-3</sup> in sampling 418 point 7, both in September 2015. Accordingly, average cell volumes in sampling points 3 and 419 7 were 0.13  $\mu$ m<sup>3</sup> and 0.30  $\mu$ m<sup>3</sup>, respectively. 420

Figure 6 presents the total number of bacteria, bacterial biomass and average cell volume 421 detected in freshwater samples in both months. It may be seen that in September 2015, in the 422 423 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 424 cell volume and bacterial biomass were similar in September 2015 in the sampling point 7. 425 Additionally, it can be observed that the average cell volume increases in areas where the 426 number of bacteria is lower both in July and September. This is due to a greater absorption of 427 nutrients by a smaller amount of microorganisms and, consequently, decreasing bacterial 428 propagation. Moreover, it can be clearly seen from the graphs that both in July and September 429 201, the total bacterial number increases in sampling point 12. Probably, the bacteria enter the 430 431 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.

436 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 441 442 during the summer and prolonged darkness during winter, persistent subfreezing temperatures, such environmental niches harbor relatively abundant life. Nonetheless, the 443 understanding of bacteria community abundance, activity and interactions with a variety of 444 polar stresses is limited. Additionally, these interactions can be fundamentally different than 445 observed elsewhere across the globe (Chu et al., 2010). Therefore it is suspected that bacterial 446 447 community in the polar regions can be considered a potential degrader of different compounds, even those regarded as anthropogenic-delivered contaminants with limited 448 biodegradation (Chu et al. 2010). 449

Concentrations of pollutants differ from each other in various aqueous reservoirs (tributaries, 450 451 lakes and river). Additionally, the interaction of hydrophobic aquatic contaminants with dissolved organic substances and particulate matter may result in the physical partitioning of 452 the compound from the water column, bringing the susceptible substrate into closer 453 association with those bacteria capable of degrading xenobiotics. Such partitioning can also 454 cause a concentration of the contaminant to toxic levels. To this point, solubilization or 455 partitioning of pollutants into dissolved organic phases may stimulate biodegradation through 456 457 availability of co-metabolizable substrates or inhibition of normal decomposition activity. 458 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 459 460 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 461 462 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 463 transported thousands of kilometers with atmospheric particles, such as desert dust, which 464 465 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 466 figure 6. In September 2015, total number of bacteria increases in almost all sampling points, 467 which can be linked to bacteria transport and their deposition with precipitation. Bacteria 468 enter the atmosphere as aerosol particles from almost all surfaces including water, soil and 469 plants (Jones and Harrison 2004). They can be removed from surfaces by gust of wind or 470 mechanical disturbances (e.g. shaking of leaves, surf breaking). Upon entering the air, 471 472 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 473 atmosphere by dry or wet deposition. The potential for bacteria and also other 474 microorganisms to be transported over long distances has fascinated microbiologists and it 475 has led to the formation of the aerobiology field. The average bacterial residence time in the 476 atmosphere may range from several days to weeks, long enough for cells to travel between 477 478 continents. Many bacterial defense mechanisms let the organisms withstand the environmental stresses of air transport, such as desiccation, exposure to UV radiation and low 479 480 pH within cloud water so some bacteria survive this long-range transport to new regions and 481 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 482 483 transported by the air and deposited in high latitudes can develop and proliferate in the Arctic (Burrows et al. 2009). 484

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<sup>-</sup>, Br<sup>-</sup> and SO<sub>4</sub><sup>2-</sup>;) and cations (Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup> and Ca<sup>2+</sup>), and the concentrations of three nutrients ions: NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup> and PO<sub>4</sub><sup>3-</sup>. 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</li>
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 495 principal components, collectively explaining 69.4 % of the variance. In the coordinate 496 system described by the two first principal components, there was a clear division between 497 samples collected in the early and late summer (Figure 8). The variables indicating chemical 498 threats and nutrients were strongly correlated, and much stronger represented in the 499 September samples. This could reflect their biological origin, as is possible in the Arctic 500 501 tundra ecosystem with a transport vector for both nutrients and pollution represented by the 502 birds feeding in the marine environment (Blais et al., 2005). In this view, the concentrations 503 of inorganic ions and PAHs cluster together, which may indicate a separate source (most 504 likely atmospheric deposition). The ammonium ion was the least correlated with the other 505 viariables (and it may indicate its spreading in the gaseous form of ammonia from the bird colonies in the vicinity, or microbial production). 506







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 516 517 environment of the Revelva catchment not to be nutrient limited, which is in accordance with 518 its rich biological life also in macroscale. These indices were equally uncorrelated with the chemical threats (pollutant concentrations), which indicates the microorganisms of the 519 520 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 521 522 they may even thrive in the environment polluted by PAHs (which is consistent with the 523 discoveries of PAH-decomposing bacteria on Arctic glaciers (Hauptmann et al., 2017). In the 524 division of sites according to hydrological environment type, the lake waters were most 525 abundant in bacteria, while streams expressed more variability in the scope of inorganic 526 chemical composition (trace elements and ions).

527 **4. Summary** 

Among polar regions, the Svalbard archipelago is distinguished by its location as the gateway 528 to the Arctic, as well as by its environmental conditions, which make it a pollution reservoir 529 (Ruman et al. 2012). Relatively close location of Svalbard archipelago to Europe makes this 530 sensitive region particularly exposed to the influence of pollutants, both emitted from 531 532 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) 533 (Kozak et al. 2016). Additionally, the landscape of Svalbard with its prevalence of mountains, 534 535 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 536 ecosystems (Laing et al. 2014; Wojtuń et al. 2013). The Revelva catchment, at the Hornsund 537 fjord, has been chosen for a comprehensive study on the pollutants deposition. Revelva 538 catchment can be specified as an ideal area to study deposition of the contaminants introduced 539 540 to the Arctic environment (Kozak et al. 2016).

The highest concentration levels of  $\Sigma$ PAHs were observed in the sample13 of July 2015and in sample 8 of September 2015, amounting to 3141.56 ng L<sup>-1</sup> and 1823.42 ng L<sup>-1</sup>, respectively. The highest concentrations of the sum of metals in both moths were found in sample 2, reaching 41.54 µg L<sup>-1</sup> in July and 79.25 µg L<sup>-1</sup> 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 547 between them is extremely difficult to study due to multiple processes occurring in the 548 environment and rapidly changing environmental conditions. It should be emphasised that 549 550 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 551 by bacteria (often called biosorption) is a mechanism that may be used to remove pollutants 552 from aqueous reservoirs. Selected features of bacteria and some special abilities such as 553 554 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 555 removal by bacteria are: biodegradation, complexation, and ion exchange. Biodegradation is 556 chemical disbanding of organic materials by bacterial communities or other biological agents 557 and involves a complex series of biochemical reactions. It usually varies with the involved 558 559 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 560 561 substances, such as phosphoric, carboxyl, phenolic, hydroxyl and sulfhydryl groups are able 562 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 563 main mode of interaction between some divalent cations and EPS (extracellular polymeric 564 substance). It has been reported that the binding between the EPS and the cations such as  $Ca^{2+}$ 565 and Mg<sup>2+</sup> is one of the main intermolecular interactions supporting bacterial communities' 566 structures. During the removal of metals by bacteria, Ca<sup>2+</sup> and Mg<sup>2+</sup> are released into the 567 water, indicating that ion exchange is involved (Wu et al. 2010a, 2011a). On the other hand, 568 in harsh polar conditions, bacterial degradation processes can be inhibited, for example due to 569 570 the lack of bioavailable nutrients or environmental stresses, hence the interactions between pollutants and bacteria abundance are fuzzy. 571

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