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10 **The interaction between bacterial abundance and selected pollutants concentration**
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,
25 when first atmospheric measurements revealed the presence of these pollutants in the polar
26 regions. Major contaminant transport routes to the Arctic include atmospheric and oceanic
27 transport, as well as inflow from rivers and sea ice. The sources of pollutants, such as
28 industry, power generators, vehicle and ship exhausts, introduce the PAHs, phenols,
29 formaldehyde or metals into the Arctic. Transport via sea currents, however, can take several
30 years. The highest concentration levels of total PAHs were observed in two samples from the
31 tributaries in July 2015 and were 1069 ng L⁻¹ and 3141 ng L⁻¹ and in September 2015, the
32 highest concentrations were observed in samples collected from Revvatnet lake and were 978
33 ng L⁻¹ and 1823 ng L⁻¹. The highest concentrations of trace elements in both months were 41

34 $\mu\text{g L}^{-1}$ in the sample from the highest tributary (July 2015) and $79 \mu\text{g L}^{-1}$ in the same sample
35 (September 2015). The purpose of this study was also to determine abundance of bacteria in
36 the Arctic freshwater of different types. Microbes are omnipresent and represent diverse
37 biological communities. In the freshwater ecosystems, microorganisms form the base of the
38 food chain supporting higher trophic levels. Although microbes are generally thought to live
39 in the warm regions of Earth, many of them develop in cold climates. In the Revelva
40 catchment, the biggest number of bacteria were detected at the river estuary in July 2015 and
41 at the sampling point located in the Revvatnet lake in September 2015. Generally, the
42 bacterial abundance indices depended on nutrient levels to a small extent, showing the
43 environment of the Revelva catchment not to be nutrient limited, which is in accordance with
44 its rich biological life also in macroscale.

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,
48 soil and sediment concentrations reveal considerably lower levels of contaminants as
49 compared to those found in temperate regions (Kallenborn et al. 2012). Despite this, some
50 characteristic features of the Arctic, e.g. low temperatures, snow precipitation, ice coverage,
51 extended periods of darkness during winter, mean that it has the potential to accumulate
52 certain globally transported contaminants including POPs (Hung et al. 2010). One of the most
53 well-known hypotheses in global atmospheric transport of POPs is the process of global
54 distillation, forcing contaminants out of the warmer regions (including temperate industrial
55 areas) into the polar areas, resulting in a complex POP distribution cycle (Kallenborn et al.
56 2015). Under ambient temperature conditions, many contaminants have partitioning
57 properties that would allow them to move from one environmental medium to another.



58 Therefore, these chemicals tend also to vaporize at elevated ambient temperature and
59 condense at lower air temperatures at higher latitudes (Kallenborn et al. 2015). In general, the
60 air masses in the temperate zones strive for a temperature balance with the cold polar air
61 masses. Due to low average annual temperature and the special seasonal daylight conditions
62 in the polar regions, the deposition of persistent pollutants is favoured there (Kallenborn et al.
63 2012). Persistent organic pollutants (POPs) are toxic substances produced by industries or
64 arising as a result of natural phenomena, and released to the environment. Within the
65 environment, POPs are resistant to degradation and may be accumulated over long periods of
66 time in liquid, solid or gas-phase reservoirs from which they enter food webs (Ma et al. 2016).
67 Persistent anthropogenic pollutants can be transported via the atmosphere, ocean currents and
68 rivers into the Arctic. After entering the polar environment, the chemicals are redistributed
69 within the region by the same transport pathways, in addition to transpolar ice transport and
70 the incorporation into the biological systems through accumulation in the food web. Each step
71 along these transport and redistribution pathways to and within the Arctic is influenced by the
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
74 reservoirs depends critically on physicochemical properties of a pollutant and environmental
75 conditions. With respect to the redistribution of POPs, important intrinsic physicochemical
76 properties including vapour pressure (P_A), Henry's Law constant (H), partition coefficients for
77 octanol-water (K_{OW}), and partition coefficients for octanol-air (K_{OA}), and susceptibility to
78 degradation or transformation (photolysis, hydrolysis, metabolism), provide kinetic controls
79 on how long a chemical may remain in any given compartment. For example, rising
80 temperatures will increase Henry's Law constant, promoting higher partitioning from water
81 into air for POPs (Ma et al. 2016). The presence of every POP in the ambient environment of
82 remote areas, such as the Arctic, shows the efficacy of atmospheric and oceanic transport to



83 these remote regions from the temperate zones, where most of toxic substances have been
84 released (Ma et al. 2016; Mackay and Wania 1995). In addition to the POPs in the Arctic,
85 there are also other chemical compounds including contaminants (e.g. metals, ions), which
86 concentration levels are visible in polar environment. The knowledge of their concentration is
87 a key element in the interpretation of the results for the presence of bacteria. Metals occur
88 naturally in the environment and are present in the soil and rocks in many forms. They can be
89 bound in organic and inorganic molecules or attached to particles in the air. Both, natural and
90 anthropogenic processes have an impact on the changes occurring in the Arctic.
91 Microorganisms depend on some metals as micronutrients but some forms of metals can be
92 toxic for them, even in relatively small amounts (Kozak et al. 2016). Other contaminants that
93 appear in the Arctic are phenols and formaldehyde. They should not be considered less
94 harmful than polycyclic aromatic hydrocarbons or polychlorinated biphenyls. Besides
95 anthropogenic sources, formaldehyde can be also emitted from the snowpack after polar
96 sunrise (Sumner et al. 2002). The low annual average temperatures of the polar regions slow
97 down the microbiological degradation of organic compounds to a minimum. That fact extends
98 the lifetime of chemicals exponentially in the Arctic (Kallenborn et al. 2015).

99 Climate change is transforming ecosystems on an extraordinary scale, and at an extraordinary
100 pace. As each species responds to its changing environment, its interactions with the physical
101 world and the organisms around it change, too. It starts a cascade of impacts throughout the
102 entire ecosystem. These impacts may include expansion of species into new areas,
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
105 polar region with impacts on species ranges, population dynamics and also food web
106 interactions (Grebmeier et al. 2006; Post et al. 2009). Despite this, little attention has been
107 given to the impacts on the microorganisms living in the Arctic. Some of these organisms

108 appear to be in a rapid decline, whereas others are shifting towards new states with
109 implications for food webs and biogeochemical fluxes including pollutants emissions. Global
110 warming leads to dwindling of the cryosphere, the ensemble of ice-containing environments
111 on the Earth. The constant decline in annual sea ice is reducing the growing season for
112 bacterial habitats that live in brine channels between the ice crystals and in the water column
113 at the edge of the observed reduction in benthic respiration rates (Grebmeier et al. 2006). It
114 may be expected that climate change will lead to the disappearance of many types of biota
115 (Vincent 2010). In the Arctic, the number of freshwater reservoirs' is increasing. Melting sea
116 ice combined with freshwater delivery from river inflows is affecting the physical
117 characteristics of the Arctic Ocean by causing reductions in the salinity of the surface mixed
118 layer, which, in turn, results in greater hydrodynamic stability of the water column. These
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,
121 erosion and greater evaporation cause some polar lakes and ponds to dry up, which leads to
122 the extinction of many bacterial communities. Furthermore, the bacterial communities
123 inhabiting freshwater ecosystems have the potential to produce globally significant effluxes of
124 greenhouse gases due to bacterial respiration and methanogenesis (Laurion et al. 2010).
125 Finally, emission of POPs to the environment can have an impact on the bacteria living in the
126 Arctic by modifying their local environment (physical aspect) and their nutrient sources and
127 interactions in the food web (biotic aspect) (Boeuf et al. 2014).

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

133 2. Materials and Methods

134 2.1. Study area

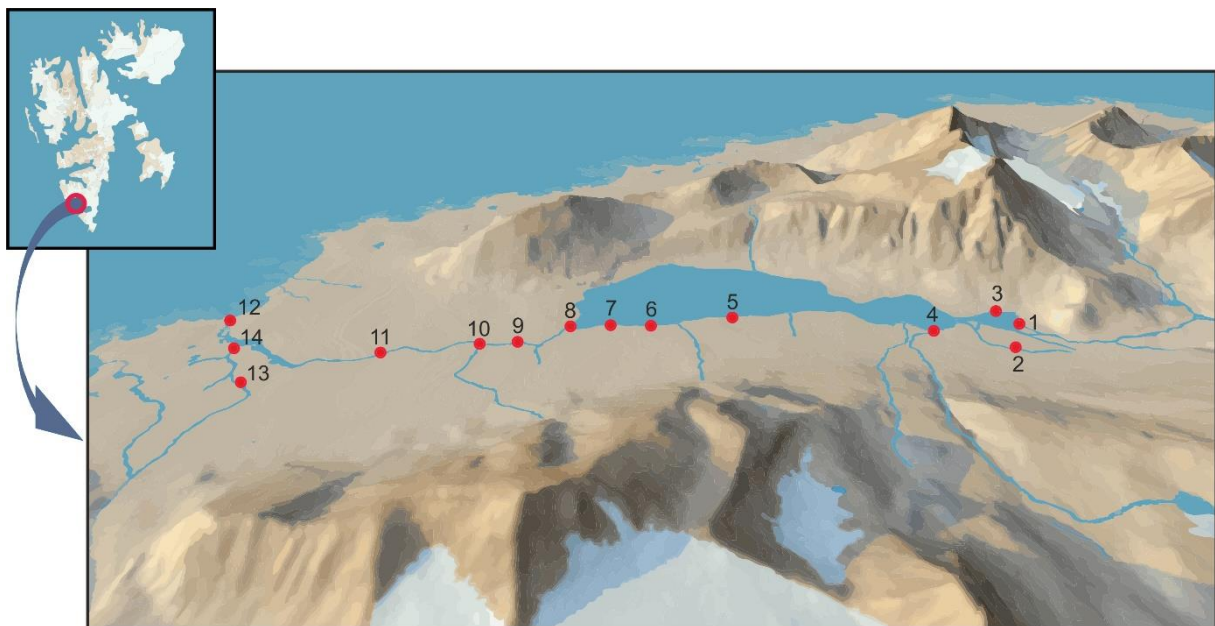
135 High latitude ecosystems may be typically thought of as extreme environments containing
136 low amount of contaminants. However, there is increasing evidence, that such a view is not
137 correct. The Svalbard archipelago, due to its specific geographical location and hydrological
138 regimes, may be defined as a place in which pollutants are permanently deposited (Kozak et
139 al. 2013). The Revelva catchment is located in the Wedel-Jarlsberg Land, in southwestern
140 Spitsbergen. The main river (Revelva) is fed both directly by atmospheric precipitation, snow
141 melt water streams and a river originating from the Arie glacier. Revelva drains into the bay
142 of Ariebukta in the south, forming an estuary. In the upper part of the catchment, the main
143 streams originate from the slopes of Eimfjellet (640 masl) and Skålfjellet (635 m asl). The
144 catchment is characterised by an asymmetry, with a predominance of left tributaries, of which
145 the proglacial Ariebekken is the largest. The sampling location has been chosen at the left
146 shore of the lake mainly to determine water input from nearby areas and the glacier. The bed
147 of Revelva is majorly paved with pebbles, with occasional boulders and sandy stretches. The
148 rocks eroded and deposited by the river belong to the Precambrian Hecla-Hoek formation
149 (Birkenmajer 1990; Czerny et al. 1993). A small part of the catchment, at the Revelva springs,
150 is built of Eimfjellet lithological groups. The Revelva catchment has only a small glacierised
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
153 terrace, areas of patterned ground and contemporary storm ridges have been formed. The
154 diversity of the catchment landscape provides an ideal setting for a comprehensive study of
155 pollutant deposition processes in different parts of the abiotic environment (Kozak et al.
156 2016).



157 2.2. Sampling

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

168



169

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

172 Freshwater samples were collected from the Revvatnet (lake) and the Revelva (river) at a
173 distance of 1.5 m from the shore with no headspace into air-tight, chemically clean 1L bottles
174 (the purity of the procedure has been verified by daily blank sample collection) using a
175 manual sampling technique. Before sampling campaign, the bottles were precisely cleaned
176 with Milli-Q deionised water (including week-long soaking and removing the water from the
177 sampling containers several times). During watercourse sampling, the neck of the bottle was
178 directed against the stream in order to prevent the inflow of suspended contaminants
179 introduced by the sampling person. The running water was taken from the main stream at
180 depths 20-50 cm below water level. For microbiological analysis, the sub-surface samples
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
184 groups and to prepare sample container blanks. The concentrations of organic and inorganic
185 compounds were determined in freshwater samples by: Inductively Coupled Plasma Mass
186 Spectrometry, Analyser TOC-VCSH/CSN with 680 °C combustion catalytic oxidation. Sum
187 of phenols and formaldehyde have been determined using Spectrophotometer - Spectroquant
188 Pharo 100 and ions have been determined using ion chromatography coupled with a
189 conductivity detector. PAHs determination was performed with a gas chromatography
190 coupled with a mass spectrometer, fitted with a detector with the electron ionization.
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.
193 Additionally, the temperature of freshwater in the Revelva catchment was measured and
194 ranged from 3 to 5 °C on the surface. Technical specifications for determining selected
195 parameters and analyte contents in the samples, and basic validation parameters of the
196 analytical procedures are summarized in Table 1.

197 **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 conductometer by 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 MSD Agilent 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 elements⁴	Li, Be, Ga, Rb, Tl,	0.010-1000	0.010	0.030	Inductively Coupled Plasma Mass Spectrometry (Thermo Scientific XSERIES 2 ICP-MS)
	U, V, Cr, Mn, Co,				
	Ni, Fe				
	B, Al, Cu, Zn, As,	0.100-1000	0.100	0.300	
	Ba				
	Sr, P	1.00-1000	1.00	3.00	

198 ¹[$\mu\text{S cm}^{-1}$], ²[mg L^{-1}], ³[ng L^{-1}], ⁴[$\mu\text{g L}^{-1}$], ⁵the limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation of the response (s)

199 and the slope of the calibration curve (b), according to the formulas: $\text{LOD}=3.3(\text{s}/\text{b})$, $\text{LOQ}=10(\text{s}/\text{b})$

200 2.4. Quality assurance / Quality Control (QA/QC)

201 The analytical procedures applied in the determination of individual components in
202 environmental samples with various matrix compositions have been validated against certified
203 reference materials. The certified reference materials (CRM) were produced and certified in
204 accordance with ISO Guide 34:2009 and ISO/IEC 17025:2005. The data obtained in the
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
207 the electrode calibration, MERCK Millipore Certipur®buffer solutions were used: pH
208 4.01/pH 7.00/pH 9.00 (25°C), which are traceable to SRM from NIST and PTB.; pH 4.01
209 (phthalate), pH 7.00 (phosphate) and pH 9.00 (borate)., The analysis of metals involved the
210 application of Standard Reference Material NIST 1643e Trace Elements in Water, and
211 Reference Material Enviro MAT ES-L-2CRM, ES-H-2 CRM SCP SCIENCE. The calibration
212 of the apparatus was based on reference materials by Inorganic ventures ANALITYK: CCS-4,
213 CCS-6, CCS-1, IV-ICPMS-71A. Potassium hydrogen phthalate by Nacalai Tesque (Japan)
214 was used for the calibration of the TOC Analyser. The sensitivity of the applied methods was
215 tested by injecting standard mixtures of the analytes in the measurement range concentration.
216 Linear calibration curves of the peak area against standard concentration showed correlation
217 coefficients (R^2) in the range of 0.898–0.999 for all standards. Each sample was analysed in
218 triplicate. During the analysis of the samples, procedural blanks (Milli-Q water with no
219 certified material addition) were prepared for every batch of six samples to check the
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
223 were in range: 70%–85% and of internal standards 80% and 85%, for naphthalene-d8 and
224 benzo[a]anthracene-d12, respectively. All blanks were analysed in the same setup as the

225 samples, using the same reagents. Additionally, formaldehyde and the sum of phenols
226 measurements have been done in accordance with ISO 8466-1 and DIN 38402 A51,
227 respectively.

228 2.5. Microbiological Analysis

229 From 1 to 5 ml of the sample has been filtered through a polycarbonate membrane filter with
230 a pore diameter of 0.2 μm and then stained with DAPI (4,6-diamidino-2-phenyl-indol) in a
231 final concentration of 2 $\mu\text{g ml}^{-1}$. The samples prepared for bacteria detection have been
232 analysed using the epifluorescence microscope Nikon Microscope 80i with NIS-Elements BR
233 3.0 and MultiScan automated image analysis system. The analysis was carried out using
234 appropriate excitation filters adapted to the used fluorochromes. The total useful microscope
235 magnification was 1200. During analysis, the image analysis system consisting of a snap-in to
236 the microscope Epifani, monochrome high resolution Nikon DS-5Mc-U2 color digital
237 camera. Structure indicators of bacteriocenosis will be estimate based on the results obtained
238 in 20 consecutive fields of view.

239 2.6. Principal Component Analysis (PCA)

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

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

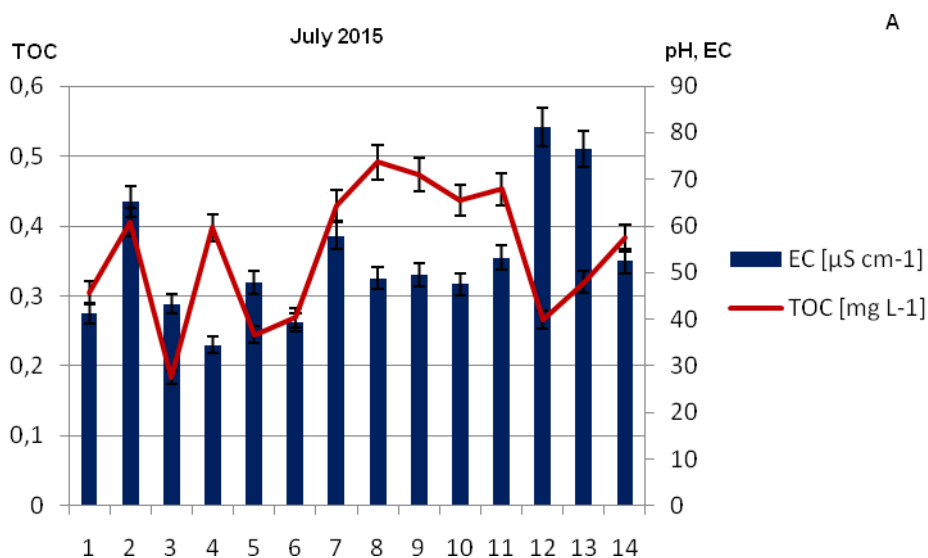
250 **3. Results and Discussion**

251 3.1. Levels of determined contaminants concentrations and other parameters of freshwater
252 samples

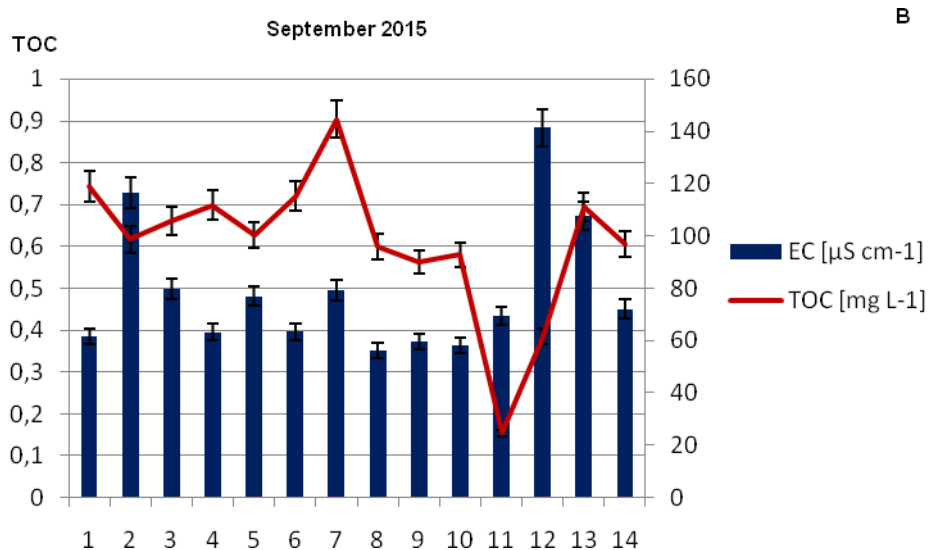
253 *Total organic carbon*

254 Total organic carbon (TOC) is the amount of carbon bound in an organic compound and it is
255 the sum of dissolved and suspended organic carbon (DOC+SOC). This constitutes a huge
256 range of compounds with a variety of properties. TOC is released to environment from both
257 natural and anthropogenic sources. All aquatic organisms release TOC through their normal
258 metabolism, excretion and eventual decomposition. Anthropogenic sources include sewage
259 treatment plants, farm slurry and silage runoff. Glacier and alpine lake ecosystems represent
260 two extremely sensitive environments to anthropogenic impacts such as inputs of dust,
261 organic matter and atmospheric pollutants which can be transported over significant distances
262 (Hood et al. 2009; Stubbins et al. 2012; Spencer et al. 2014). Total organic carbon, electrical
263 conductivity and pH have been measured. Carbon is one of the most significant component of
264 any environment, including the polar environment, characterised by an extremely harsh
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,
268 2009). The global climatic changes have significantly affected the cryosphere in many regions
269 of the world, including Svalbard Archipelago (Wang et al. 2013). Glaciers are considered key
270 indicators of climate change due to their rapid reaction to even small climatic shift through
271 melt and mass loss. This could also release pollutants from glaciers to other polar

272 environment reservoirs, as it was mentioned before. Global model simulations indicate that
 273 climatic warming is more pronounced at high latitudes in the northern hemisphere and suggest
 274 the arctic carbon cycle is extremely sensitive to climate change (Anisimov et al. 2007;
 275 Yoshitake et al. 2011). Various studies for the terrestrial Arctic show that land areas are a sink
 276 for approximately 300-600 million tonnes of carbon per year. 40-84 million tonnes of carbon
 277 is released to the atmosphere from lakes and rivers each year and seawater appears to be a
 278 sink for $24-100 \cdot 10^{12} \text{ gC yr}^{-1}$. Carbon is also carried from land to rivers and from rivers to
 279 oceans and then it can be emitted to the atmosphere or captured in sediments (McGuire et al.
 280 2009; AMAP 2009). In the collected freshwater samples, TOC ranged from 0.184 ± 0.010 to
 281 $0.491 \pm 0.010 \text{ mg L}^{-1}$ in July and from 0.154 ± 0.010 to $0.903 \pm 0.040 \text{ mg L}^{-1}$ in September (Fig.
 282 2A,2B).



283



284

285

286

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

289 Transport and fate of TOC in aquatic systems are both determined by the local patterns of
 290 precipitation, physical and biological interactions, runoff and outflows from glaciers (Hood et
 291 al. 2003). Taking into consideration, average results of precipitation collected in July (7.9
 292 mm), August (73.0 mm) and September (171.4 mm), it may be stated that large amount of
 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
 296 from within the aquatic ecosystem, derived from algal and bacterial biomass. Relative balance
 297 of all TOC sources in aquatic ecosystems may affect the aquatic biota by altering the amount
 298 of energy available for heterotrophic growth (Hood et al. 2003).

299 Besides TOC measurements, electrical conductivity and pH have been checked in freshwater
 300 samples collected in 2015. The hydrochemical studies of polar areas were carried out over

301 many years in the surroundings of the Hornsund fjord, also in the catchment of Revelva, and
 302 demonstrated high hydrochemical variability. Nevertheless, some of the parameters have been
 303 marked at similar ranges, for example, all samples collected in previous years, and also these
 304 collected in July and September 2015, were characterised by slightly acidic pH (Fig 2A, 2B)
 305 (Ruman et al. 2012; Kozak et al. 2016).

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

313 *Organic compounds*

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

318 **Table 2.** Concentration levels (\pm standard deviation, SD) of PAHs, sum of phenols and
 319 formaldehyde determined in the collected freshwater samples.

		July 2015	September 2015
PAHs	Naphthalene	108 \pm 11 — 518 \pm 40	76.1 \pm 9.6 — 1823 \pm 41
[ng L ⁻¹]	Acenaphthylene	1.30 \pm 0.18 — 52.5 \pm 8.7	0.70 \pm 0.41 — 17.6 \pm 1.6



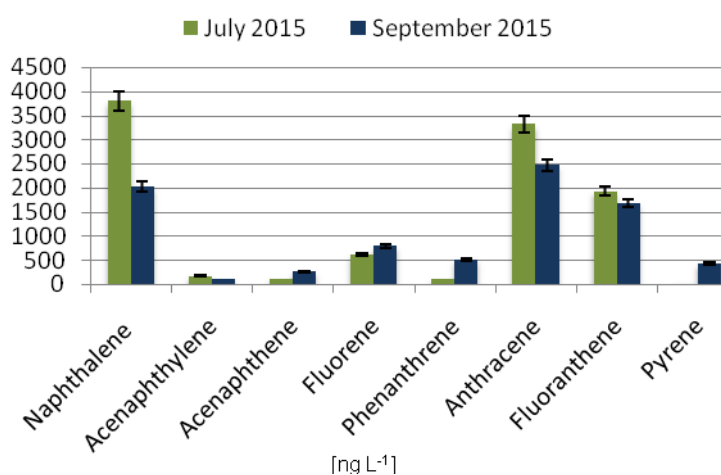
	Acenaphthene	0.75±0.32 — 44±16	3.80±0.23 — 146±26
	Fluorene	3.8±2.9 — 219±31	5.80±0.70 — 986±29
	Phenanthrene	9.7±7.0 — 29±12	0.70±0.30 — 58.3±8.6
	Anthracene	21±12 — 1450±59	23.7±4.9 — 1342±42
	Fluoranthene	6.6±1.2 — 853±81	6.90±0.91 — 898±24
	Pyrene	2.14±0.21 — 14.3±9.7	2.10±0.21 — 244±21
	Phenolic compounds,		
	Formaldehyde		
	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

320

321 Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals that are
322 formed during the incomplete burning of coal, biomass, fossil fuels and other industrial
323 activities. They have been found in the Arctic environment, and the long-range atmospheric
324 transport is believed to be one of their primary entry routes (Wang et al. 2013). Alongside the
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
327 carcinogenic, mutagenic and toxic contaminants. PAHs have been found widely in polar
328 environmental media, such as atmosphere, water, ice, snow, sediments and biota (Polkowska
329 et al. 2011; Kozak et al. 2017). They can be deposited and accumulated in ice for a long
330 period of time and released to the environment when temperature exceeds the melting point
331 (Ge et al. 2016). The results of PAHs analysis are shown in Table 2. The highest
332 concentration levels of PAHs have been detected in the sampling point 13 in July and in the

333 sampling point 8 in September, and these were $1450 \pm 59 \text{ ng L}^{-1}$ and $1342 \pm 42 \text{ ng L}^{-1}$,
334 respectively.

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



343
344 **Figure 3.** The average concentration levels of the determined PAH congeners in freshwater
345 samples collected in July and September 2015.

346 *Trace elements*

347 Trace elements were also determined in the samples collected in July and September 2015
348 from the Revelva catchment. The concentrations of the following trace elements were
349 determined in the collected samples: Li, Be, B, Al, P, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, As,

350 Rb, Sr, Ba, Tl, and U (Table 3). The concentration levels of the trace metals detected in
 351 freshwater ranged from 12.4 to 41.5 $\mu\text{g L}^{-1}$ in July 2015 and from 36.2 to 80.9 $\mu\text{g L}^{-1}$ in
 352 September 2015. Metals concentration CV ranged from 0.5 to 1.5%.

353 **Table 3.** Concentration levels (\pm standard deviation, SD) of trace elements in the collected
 354 freshwater samples.

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

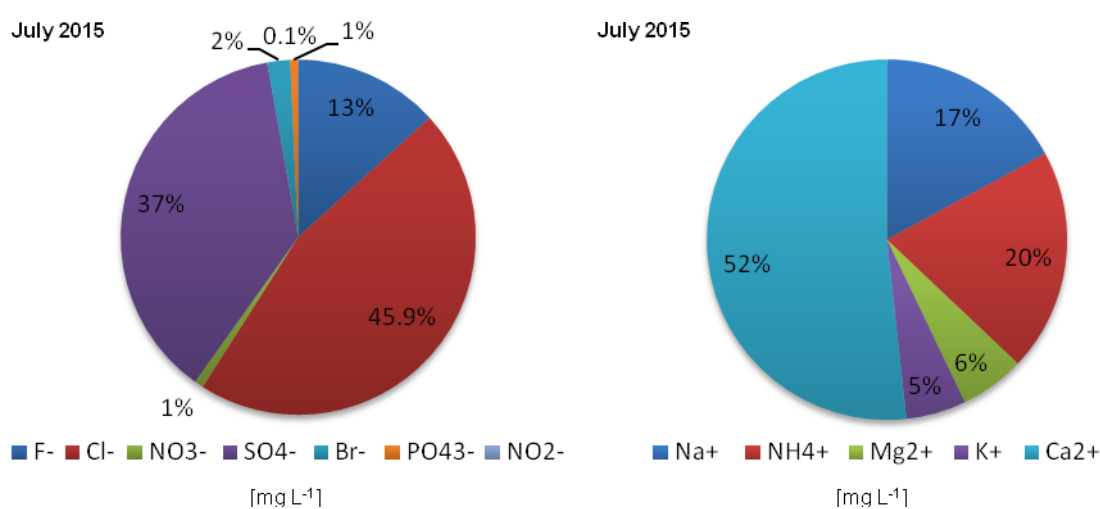
Ba	1.03±0.19 — 5.9±1.2	1.15±0.12 — 11.8±8.3
Tl	0.010±0.011	0.010±0.011 — 0.040±0.016
U	0.010±0.010 — 0.04±0.015	0.010±0.010 — 0.98±0.47

355

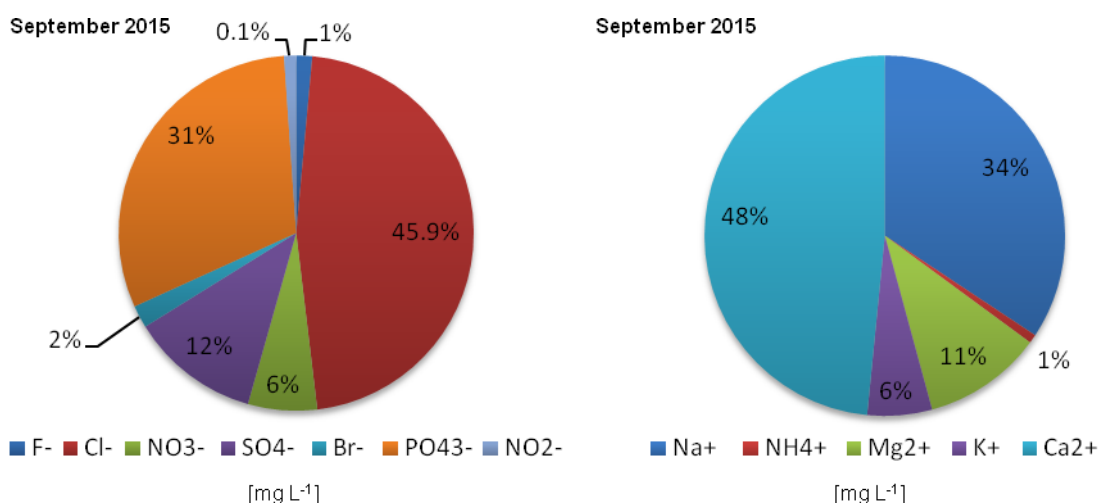
356 The concentrations detected in two different months varied from each other significantly. The
357 increase of trace element concentrations in September can be well observed. This may be
358 caused both as a result of intensive precipitation in September and consequently greater wet
359 deposition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

375 *Ions*

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



392



393

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

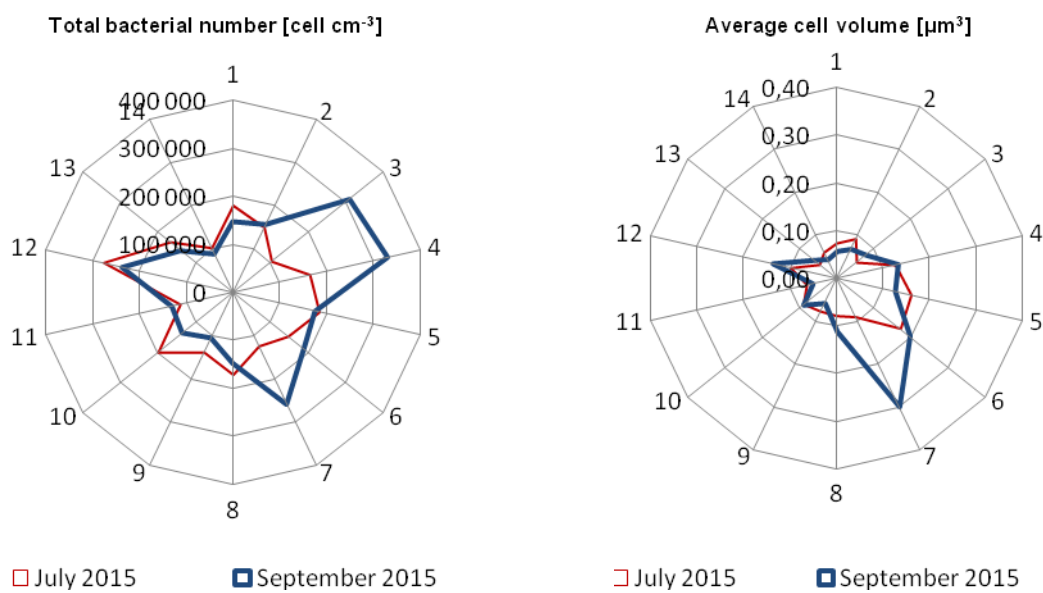
395 3.2. Results of the microbiological analysis

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

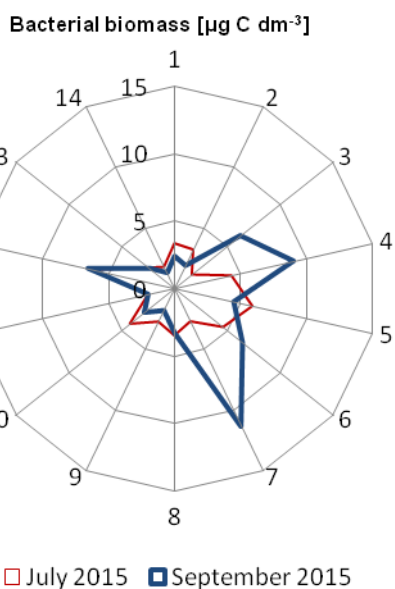
408 The biomass of bacterial population has been recognised as an important parameter when
 409 studying microbial ecology. An estimate of microbial production can be used as a general

410 index of microbial activity and specifically to calculate growth rates. Since many processes
411 scale with it, biomass production can be used to obtain a first-order estimate of rates of
412 several processes mediated by microbes. For example, in case of heterotrophic bacteria, which
413 are the subject of this study, biomass production can be used to estimate the use of dissolved
414 organic carbon if coupled with an estimate of the growth efficiency (Kirchman 2001). In
415 collected freshwater samples, higher bacterial biomass was detected in September 2015,
416 especially in the sampling points 4 and 7. It is strongly linked to average bacterial cell
417 volumes which at these points were also bigger. In the collected samples, the highest values of
418 bacterial biomass were $9.05 \mu\text{g C dm}^{-3}$ in sampling point 4 and $11.37 \mu\text{g C dm}^{-3}$ in sampling
419 point 7, both in September 2015. Accordingly, average cell volumes in sampling points 3 and
420 7 were $0.13 \mu\text{m}^3$ and $0.30 \mu\text{m}^3$, respectively.

421 Figure 6 presents the total number of bacteria, bacterial biomass and average cell volume
422 detected in freshwater samples in both months. It may be seen that in September 2015, in the
423 sampling points 3, 4 and 7, bacteria were more abundant than in July 2015. It is caused by
424 greater nutrients bioavailability and probably less exposure to stress factors. Average bacterial
425 cell volume and bacterial biomass were similar in September 2015 in the sampling point 7.
426 Additionally, it can be observed that the average cell volume increases in areas where the
427 number of bacteria is lower both in July and September. This is due to a greater absorption of
428 nutrients by a smaller amount of microorganisms and, consequently, decreasing bacterial
429 propagation. Moreover, it can be clearly seen from the graphs that both in July and September
430 201, the total bacterial number increases in sampling point 12. Probably, the bacteria enter the
431 estuary along with the marine waters of the Hornsund Fjord.



432



433

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

436 3.3. Selected chemical compounds and their influence on bacterial abundance

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

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

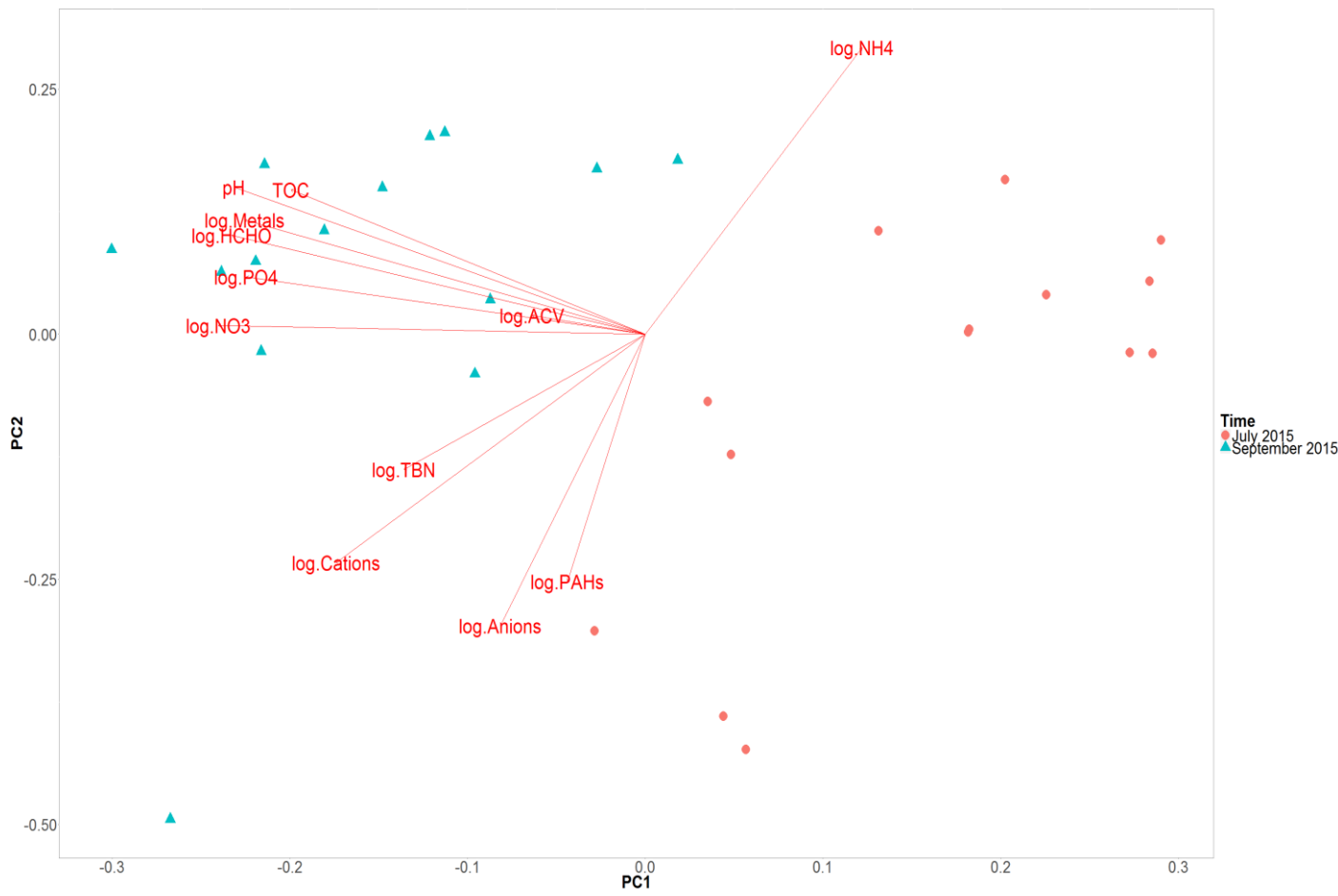
450 Concentrations of pollutants differ from each other in various aqueous reservoirs (tributaries,
451 lakes and river). Additionally, the interaction of hydrophobic aquatic contaminants with
452 dissolved organic substances and particulate matter may result in the physical partitioning of
453 the compound from the water column, bringing the susceptible substrate into closer
454 association with those bacteria capable of degrading xenobiotics. Such partitioning can also
455 cause a concentration of the contaminant to toxic levels. To this point, solubilization or
456 partitioning of pollutants into dissolved organic phases may stimulate biodegradation through
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
459 or increase the solubility of polycyclic aromatic hydrocarbons (Wu et al. 2010a, 2011a). On
460 the other hand, in aquatic environment, there are some bacterial communities incapable of
461 degrading pollutants, and in such areas the concentration levels of contaminants increase
462 significantly. Moreover, an unexpected growth of bacterial communities in the aquatic
463 reservoirs may be also result from bacterial deposition from the atmosphere. Bacteria can be
464 transported thousands of kilometers with atmospheric particles, such as desert dust, which
465 influences atmospheric chemistry, microbial biogeography and the global distribution of

466 clouds and precipitation (Burrows et al. 2009). For example, this trend can be seen in the
467 figure 6. In September 2015, total number of bacteria increases in almost all sampling points,
468 which can be linked to bacteria transport and their deposition with precipitation. Bacteria
469 enter the atmosphere as aerosol particles from almost all surfaces including water, soil and
470 plants (Jones and Harrison 2004). They can be removed from surfaces by gust of wind or
471 mechanical disturbances (e.g. shaking of leaves, surf breaking). Upon entering the air,
472 bacteria may be transported upwards by the air currents and due to their size, remain in the
473 atmosphere for an average period of a few days. They are eventually removed from the
474 atmosphere by dry or wet deposition. The potential for bacteria and also other
475 microorganisms to be transported over long distances has fascinated microbiologists and it
476 has led to the formation of the aerobiology field. The average bacterial residence time in the
477 atmosphere may range from several days to weeks, long enough for cells to travel between
478 continents. Many bacterial defense mechanisms let the organisms withstand the
479 environmental stresses of air transport, such as desiccation, exposure to UV radiation and low
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
482 only bacteria that multiply in polar regions are encountered there. Those bacteria which are
483 transported by the air and deposited in high latitudes can develop and proliferate in the Arctic
484 (Burrows et al. 2009).

485 To explore patterns in the variability of the chemical background and bacterial abundance, we
486 have conducted principal component analysis (PCA) on a set of chosen variables: TBN, ACV,
487 TOC, pH, sum of PAHs, the concentration of formaldehyde (HCHO), the sum of
488 concentration of chosen trace elements, i.e.: Li, Be, B, Al, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga,
489 As, Rb, Sr, Ba, Tl, and U (Metals), sums of anions (F^- , Cl^- , Br^- and SO_4^{2-} ;) and cations (Na^+ ,
490 K^+ , Mg^{2+} and Ca^{2+}), and the concentrations of three nutrients ions: NO_3^- , NH_4^+ and PO_4^{3-} . BB

491 was not included as a variable derived from TBV and ACV, and sum of phenols was omitted
492 to due to overrepresentation of values below LOD. The other <LOD values were replaced by
493 0.5 LOD. All variables except TOC and pH were log-transformed to reflect the nature of their
494 distribution.

495 Based on the eigenvalue >1 criterion, we have distinguished three potentially significant
496 principal components, collectively explaining 69.4 % of the variance. In the coordinate
497 system described by the two first principal components, there was a clear division between
498 samples collected in the early and late summer (Figure 8). The variables indicating chemical
499 threats and nutrients were strongly correlated, and much stronger represented in the
500 September samples. This could reflect their biological origin, as is possible in the Arctic
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 variables (and it may indicate its spreading in the gaseous form of ammonia from the bird
506 colonies in the vicinity, or microbial production).



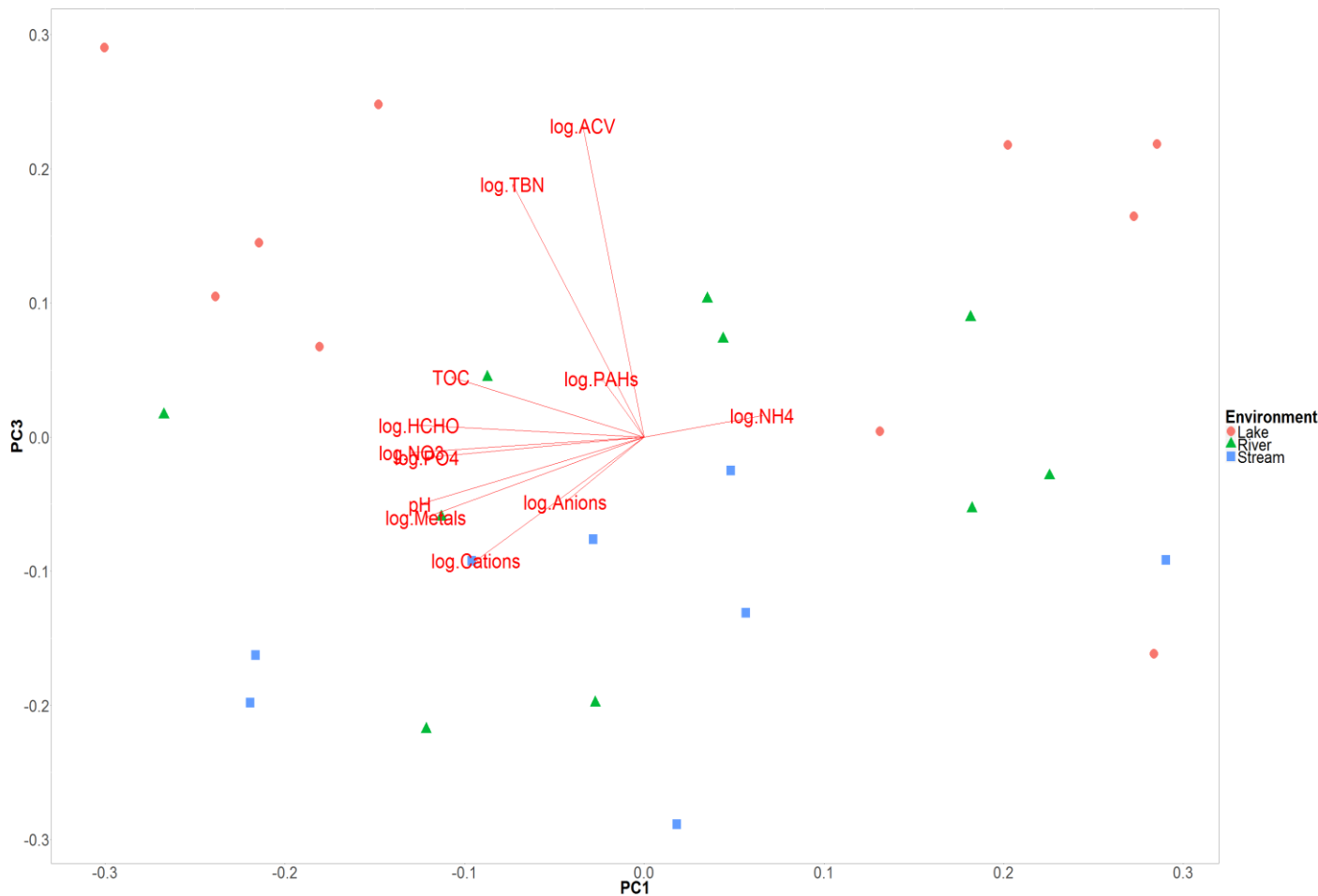
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513 **Figure 8.** Variables as vectors in the space defined by three principal components described
 514 in text (from top to bottom: F1/F2 and F1/F3); points are samples (representing 14 different
 515 sites and two dates).

516 The bacterial abundance indices depended on nutrient levels to a small extent, showing the
 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
 519 chemical threats (pollutant concentrations), which indicates the microorganisms of the
 520 Revelva catchment cope well with the experienced levels of pollution. An exception is
 521 perhaps the concentration of PAHs, positively correlated with bacterial indices, which shows
 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**

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

541 The highest concentration levels of Σ PAHs were observed in the sample 13 of July 2015 and in
542 sample 8 of September 2015, amounting to 3141.56 ng L⁻¹ and 1823.42 ng L⁻¹, respectively.
543 The highest concentrations of the sum of metals in both months were found in sample 2,
544 reaching 41.54 μ g L⁻¹ in July and 79.25 μ g L⁻¹ in September. The concentration levels of the
545 sum of phenols and formaldehyde were two times higher in September than in July. It results
546 from more frequent precipitation in September and the wet deposition of these pollutants.



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

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