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2 Postprint of: Kosek K., Koziół K., Łuczkiwicz A., Jankowska K., Chmiel S., Polkowska Ż., Environmental
3 characteristics of a tundra river system in Svalbard. Part 2: Chemical stress factors, SCIENCE OF THE
4 TOTAL ENVIRONMENT, Vol. 653 (2019), pp. 1585-1596, DOI: [10.1016/j.scitotenv.2018.11.012](https://doi.org/10.1016/j.scitotenv.2018.11.012)

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

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23

24 **Abstract:** Bacterial communities in the Arctic environment are subject to multiple stress
25 factors, including contaminants, although typically their concentrations are small. The Arctic
26 contamination research has focused on persistent organic pollutants (POPs) because they are
27 bioaccumulative, resistant to degradation and toxic for all organisms. Pollutants have entered
28 the Arctic predominantly by atmospheric and oceanic long-range transport, and this was
29 facilitated by their volatile or semi-volatile properties, while their chemical stability extended
30 their lifetimes following emission. Chemicals present in the Arctic at detectable and
31 quantifiable concentrations testify to their global impact. Chemical contamination may induce
32 serious disorders in the integrity of polar ecosystems influencing the growth of bacterial

33 communities. In this study, the abundance and the types of bacteria in the Arctic freshwater
34 were examined and the microbial characteristics were compared to the amount of potentially
35 harmful chemical compounds in particular elements of the Arctic catchment. The highest
36 concentrations of all determined PAHs were observed in two samples in the vicinity of the
37 estuary both in June and September 2016 and were 1964 ng L⁻¹ (R12) and 3901 ng L⁻¹ (R13)
38 in June, and 2179 ng L⁻¹ (R12) and 1349 ng L⁻¹ (R13) in September. Remarkable
39 concentrations of the sum of phenols and formaldehyde were detected also at the outflow of
40 the Revelva river into the sea (R12) and were 0.24 mg L⁻¹ in June and 0.35 mg L⁻¹ in
41 September 2016. The elevated concentrations of chemical compounds near the estuary
42 suggest a potential impact of the water from the lower tributaries (including the glacier-fed
43 stream measured at R13) or the sea currents and the sea aerosol as pollutant sources. The
44 POPs' degradation at low temperature is not well understood but bacteria capable to
45 degrading such compounds were noted in each sampling point.

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

48 **1. Introduction**

49 The Arctic contains some highly productive ecosystems despite its extreme environmental
50 conditions, strong seasonal changes in irradiance and snow cover, and the primary
51 productivity concentrated in the short summer (Nguyen et al. 2015). Bacterial extremophiles
52 are among the dominant life forms in the Arctic. They are able to survive in the harsh polar
53 conditions and have developed mechanisms that allow them to cope with a variety of stress
54 factors, e.g. temperature fluctuations, repeated freeze-thaw cycles, high or low levels of
55 salinity or pH, UV light and desiccation (Sahay et al. 2013; Hoover and Pikuta 2013;
56 Ntougias et al. 2016). These environmental stresses are yet enhanced by the increasing



57 concentrations of harmful chemical compounds, including persistent organic pollutants
58 (POPs). There are a few local sources of contaminants in the Arctic, such as military
59 installations, industrial outlets and waste from the old mines, settlements and ships, or the use
60 of insecticides for insect control. However, the majority of Arctic pollution problem arises
61 from a combination of long-range transport of pollutants and the Arctic haze phenomenon,
62 locking the contaminated air in the area for months.

63 The concentrations of chemical compounds, including contaminants, differ in various aqueous
64 reservoirs: lakes, river and tributaries (Kosek et al. 2018). Pollutants in the environment are
65 exposed to degradative forces. Among them biotic degradation or metabolic processes are
66 known to play a vital role in deciding overall fates of organic pollutants. They not only
67 contribute to the disappearance of the original form of pollutants but also change their
68 physicochemical properties and due to it, their transport and distribution behavior among
69 various compartments in the environment. Physical and chemical factors may render a given
70 contaminant more or less susceptible to bacterial degradation (Matsumura 1989). On the other
71 hand, in aquatic environment, there are some bacterial communities incapable of degrading
72 pollutants, and in such areas the concentration levels of pollutants increase remarkably (Ma et
73 al. 2016; Nadal et al. 2015). Lakes in remote areas such as the Arctic have been of particular
74 interest over the last decade for investigating the fate and dynamics of POPs (Evenset et al.
75 2004; Evenset et al. 2007; Ahrens et al. 2016). Increasing trends in contamination levels
76 suggest that these areas are significant trapping sites of persistent toxic pollutants (Jiao et al.
77 2009). Due to the low temperature in the Arctic, mineralization of POPs is extremely slow in
78 cold habitats and they likely bioaccumulate in the adipose tissue and then biomagnify in
79 species inhabiting the polar regions (Kosek et al. 2007). Bacteria inhabiting the Arctic, are
80 more strained and susceptible to the adverse effects of POPs than the bacteria living in other
81 regions due to their long life and slow detoxifying. Moreover, in the nutrient-limited

82 environments, aromatic compounds may serve as a carbon source, also under sulfate-reducing
83 and nitrate-reducing conditions. However, very little is known about their anaerobic
84 degradation pathways (Foght 2008; Mallick et al. 2011), particularly in polar regions. Low
85 temperature catabolic genes/enzymes activity is of a great interest due to the their
86 biotechnological applications.

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

93 **2. Materials and Methods**

94 2.1. Fieldwork

95 The study was conducted in the Revelva catchment (Wedel-Jarlsberg Land, southwestern
96 Spitsbergen, near the Polish Polar Station Hornsund). A detailed map of the sampling area is
97 shown in Part 1 of this article (Kosek et al. submitted) and our former work (Kosek et al.
98 2018). In brief, the samples were taken from 14 locations in the river, its tributaries and lakes
99 through which it flows, from mountain streams filling rocky beds to its estuary at the
100 Hornsund fjord bay Ariebukta (Table 1). Among the tributaries, the largest one was fed by
101 glacier melt (Ariebekken). Each place was sampled twice, in June and September 2016,
102 reflecting a shift from melting snow patches to permafrost thaw and rainwater as main water
103 sources over the summer season. Furthermore, three points (R4, R8 and R14) were checked
104 for the bacterial taxonomy. The tested points differed remarkably in terms of geological
105 substratum, vegetation and water flow velocity, which influence chemical constituent sources

106 and the potential of self-cleaning for these environments. Points R4 and R8 were located in
107 the areas of no or limited biological soil crust, while the point R14 was surrounded by boggy
108 vegetation, composed of a mixture of mats formed by cyanobacteria and bryophytes, as well
109 as by small lichens and saxifrages in varying proportions (Kumar et al. 2017). Additionally, it
110 should be noted that water was flowing in the points R8 and R14 (most rapidly in R8), while
111 in the point R4 (lake) it was relatively stagnant. Separate aliquots were prepared for chemical
112 composition analysis (in pre-cleaned 1 L HDPE bottles, stored at 4°C), microbiological
113 parameters quantification (50 mL, preserved with 2% formaldehyde, stored at 4°C) and
114 metagenomics (1.5 L, stored frozen).

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

116 2.2. Chemical Analysis

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

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

127 2.3. Quality assurance / Quality Control (QA/QC)

128 The analytical procedures used to determine individual components in the studied samples
129 have been validated against certified reference materials (CRMs) concordant with ISO Guide
130 34:2009 and ISO/IEC 17025:2005. The data obtained here were subject to strict QC
131 procedures. The analysis of trace elements involved the application of Standard Reference
132 Material (RM) NIST 1643e Trace Elements in Water, and RM Enviro MAT ES-L-2CRM,
133 ES-H-2 CRM SCP SCIENCE. The calibration of the apparatus was based on RMs by
134 Inorganic ventures ANALITYK: CCS-4, CCS-6, CCS-1, IV-ICPMS-71A. The sensitivity of
135 the applied methods was tested by injecting standard mixtures of the analytes in the measured
136 concentration range. Linear calibration curves of the peak area against standard concentration
137 showed correlation coefficients (R^2) in the range of 0.898–0.999 for all standards.
138 Technically, each sample was analysed in triplicate. The instrumental background was
139 checked by inserting Milli-Q water blanks once per every six samples. All the obtained values
140 for organic compounds (PAHs) in CRMs were within the confidence interval. Reproducibility
141 and recovery for both groups of organic compounds were high (85%–105%) with relative
142 standard deviation (RSD) 4%–10%. Finally, the measurements of formaldehyde and the sum
143 of phenols have been done in accordance with norms ISO 8466-1 and DIN 38402 A51,
144 respectively.

145 2.4. Bacterial Abundance Analysis

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

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

154 2.5. Bacterial Community Structure Analysis

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

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

175 3. Results and Discussion



176 3.1. Chemical stress factors occurring in the studied freshwater samples

177 3.1.1. pH

178 In freshwater environments, pH has been shown to be a decisive environmental factor
179 determining the bacterial community composition, often being the most important one
180 compared to factors such as temperature, organic matter, water retention time, and nutrient
181 concentrations (Lindström et al. 2005). pH is also an environmental factor that can vary
182 greatly in aquatic ecosystems (Bååth and Kritzberg 2015). Lake, river and stream waters can
183 have pH values below 4 and above 9 even within small geographical areas. In highly
184 productive lakes, pH at the surface may be 2 units higher than in bottom waters. The variation
185 of the values is driven by vertical differences in photosynthesis, respiration, and redox
186 conditions (Wetzel 2001). pH can also fluctuate rapidly. For example, during snow melt and
187 rain storms, pH values in streams can decrease several units, sometimes within a few hours
188 (Lawrence 2002). On the other hand, sunny days can result in high photosynthetic activity
189 with the increase of water pH values. Accordingly, changes of 2-3 pH units may be found in
190 highly productive aquatic environments (Tank et al. 2009). During episodes of rapid pH
191 changes, the bacterial community may not be optimally adapted to the new pH condition,
192 resulting in impaired functions, and sometimes, inhibition of bacterial growth (Bååth and
193 Kritzberg 2015). Freshwater pH values, and in particular their changes, may pose a big threat
194 to bacterial development and play a key role as a stress factor. However, in our study, the pH
195 values in the collected samples were differing only slightly and ranged from 7.0 to 8.0 both in
196 June and September 2016 (Figure 1). Former hydrochemical studies of the Hornsund fjord
197 area (including Revelva catchment) show high hydrochemical variability, with some values
198 within similar ranges as described in Part 1 of this article (Kosek et al. submitted). Small pH
199 values variation can be explained by the ability of bacteria to regulate the pH of water.
200 Consequently, bacteria are able to survive and develop even in the most harsh conditions. The

201 interaction between the microbes may be set by how their metabolism change the
202 environment and react to those changes. Furthermore, many biochemical reactions involve a
203 turnover of protons and bacteria also alter the pH around them. When the pH modification is
204 beneficial for the bacteria, there is a positive feedback on their growth. The more bacteria
205 there are in the water, the stronger they can change the environment. At adverse pH
206 conditions, a sufficiently high cell density may therefore be needed to survive at all (Ratzke
207 and Gore 2018).

208 **Figure 1.** The pH values detected in freshwater samples collected in June and September
209 2016.

210 3.1.2. Trace elements

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

215 **Table 3.** Concentrations (\pm standard deviation, SD) of trace elements in the collected
216 freshwater samples.

217 The Revelva catchment waters are enriched in trace elements due to the presence of ore-
218 bearing veins and metamorphic rocks in the area (Wojciechowski 1964; Smulikowski 1965).
219 This geological substratum is more exposed in the upper parts of the catchment. Furthermore,
220 the spatial variability of the underlying rocks in this catchment allows for the more abundant
221 occurrence of titanium, possibly also barium, caesium, lithium, rubidium, and zinc in the
222 upper part of the catchment; of zirconium in the left tributaries of the middle part, and of
223 chromium and vanadium in both these areas. The local rocks are relatively abundant in
224 aluminum and manganese throughout the catchment (Smulikowski 1965). As for the ore-

225 bearing veins, in the area occur those with chalcopyrite, cuprite, malachite and azurite, which
226 are copper minerals, as well as smaller concentrations of sphalerite (with zinc) and galena
227 (with lead). The specific locations of these veins favour the occurrence of copper near
228 Skoddefjellet mountain, in the Arie glacier valley, and in the left tributaries of the biggest and
229 smallest lakes in the valley (the top nameless lake and Revvatnet), the occurrence of lead in
230 the Arie glacier valley and of both lead and zinc in the left tributaries of the smallest lake
231 (Wojciechowski, 1964).

232 The trace metal concentrations detected at the two sampling occasions markedly differed from
233 each other, with an increase in September. This may be caused by the occurrence of
234 groundwater associated with the active layer of permafrost, which gains more importance in
235 the hydrological regime of Revelva as snow patches disappear in the catchment. Apart from
236 the local natural occurrence of trace elements, they are assumed to be derived to the Arctic
237 mostly from long-range atmospheric transport (AMAP 2009), and these may be additionally
238 supplied by September rainfalls. The increase in concentration of trace elements in September
239 2016 was most evident in the central part of the lake shore and in two points located near the
240 river estuary. The water stagnating in the lake experiences longer contact with suspended
241 mineral matter, hence probably the higher trace element concentrations there. A similarly
242 longer time may have contributed to the higher concentrations near the river mouth.
243 Differences in individual trace element concentrations can be explained qualitatively in terms
244 of mineral surface reactions, complexation, chemical weathering and sorption to solid-phase
245 soil organic matter (Colombo et al. 2018), yet the detailed extent of these processes cannot be
246 determined with the limited data we obtained and it is outside the scope of the current paper.

247 3.1.3. Organic compounds

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

251 **Table 4.** Concentrations (\pm standard deviation, SD) of PAHs, formaldehyde and the sum of
252 phenols in the collected freshwater samples.

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

270 The sampling point R13 is located at the outflow from the Arie glacier, thus such a high
271 concentration of PAHs observed in June can be explained by releasing pollutants from the

272 melting snow cover of the glacier. The difference between PAHs composition in June and
273 September in the catchment (Figure 2) reflects well the order of PAHs elution from melting
274 snowpack and the preferential storage of the more hydrophobic PAHs in ice (Kozioł et al.
275 2017).

276 **Figure 2.** A box - whisker plot of the mean PAHs concentrations in water samples collected
277 in June and September. Significant differences between seasons are indicated with the non-
278 parametric Kruskal-Wallis ANOVA p-levels below 0.05 and given in boldface. The box
279 encompasses the mean value \pm SD, the whiskers show the full range of values noted (where
280 $<$ LOD values were assigned a half of the LOD level).

281 3.2. Microbial community

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

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

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

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

313 Major PAHs degrade approaches are highly linked to the oxygen presence/absence. Under
314 aerobic conditions the oxygen is both the final electron acceptor and co-substrate to activate
315 and subsequently cleave the aromatic ring, catalyzed by oxygenase enzymes (monooxygenase
316 or dioxygenase) (Foght 2008; Carmona et al. 2009). In the anoxic conditions, which is
317 regarded as more common in natural environments (e.g. aquifers, aquatic sediments and
318 submerged soils), the attack on the aromatic ring is primarily based on reductive reactions
319 (Foght 2008; Carmona et al. 2009), where nitrate, sulfate or ferric ions are used as final
320 electron acceptors (Foght 2008; Carmona et al. 2009). For instance, *Hyphomonas* detected in

321 each sampling point in this study (see Table 5), showed the degradation potential after the
322 addition of sources of nitrogen and phosphate to hydrocarbon-contaminated harbour
323 sediments (Yakimov et al. 2005). The metabolic pathways of sulfate-reducing and Fe(III)-
324 reducing bacteria are also of interest due to the role they play in the biogeochemistry
325 including degradation of organic contaminants (Meckenstock et al. 2000). The sulfate-
326 reducing *Desulfovibrio*-like bacteria, forming up to 0.26% of the bacterial community
327 composition in the studied samples, as well as the spore-forming *Desulfotomaculum* and
328 *Desulfosporosinus* genera from *Firmicutes* phylum (forming up to 0.22 % of the bacterial
329 community), are also potentially capable of decomposing the organic pollutants occurring in
330 this catchment, since these bacterial groups may use a variety of aliphatic and aromatic
331 compounds as a carbon source (Hansen 1994). Interesting is also high relative abundance of
332 psychrotolerant *Rhodoferrax* genus (from 1.56% to 5.6%), already reported as potential
333 phenanthrene degrader (Martin et al., 2012), and in this study represented mainly by *R.*
334 *ferrireducens* (>85%), which is capable of dissimilatory Fe(III) reduction at low temperature.

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

345 **Table 5.** The list of members of bacterial community possibly involved in the degradation of
346 pollutants. A genus was considered only if it constituted $\geq 0.01\%$ of the community from a
347 single sample.

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

353 3.3. Statistical analysis of bacterial abundance and chemical background in the Revelva 354 catchment

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

367 In the analysis conducted for the whole summer season, the scree plot shape suggested that
368 the PCs 1, 2 and 3 were likely significant factors. We also conducted further analyses for June



369 and September separately, showing only the first two PCs in each (also as suggested by the
370 scree plot)

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

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

390 On the other hand, the late season was characterised by a higher concentration of most other
391 toxic elements and compounds and the TBN index, which shows that the r-strategists were not
392 limited by these toxic chemicals in their reproduction. This can be interpreted in terms of the

393 concentrations being too low to have a limiting impact on the bacterial community.
394 Furthermore, some of the detected trace elements (such as Cu, Mn and Zn) have ample local
395 sources in the geological substratum and therefore the local bacterial community is well
396 adjusted to their presence. It is interesting to notice, however, that the most abundant presence
397 of zinc deviates from its naturally enriched area in the upper part of the catchment (with
398 higher concentrations in some of the samples located in the lower parts of the catchment,
399 especially points R10 and R13), which may suggest its pollution origin. It is also the only
400 trace metal in this analysis to correlate closer with ACV than TBN.

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

414 The type of hydrological environment was best distinguished on the PC 2 and 3 graph (Figure
415 3. Bottom), depicting approximately 27% of the total variability in the dataset. The river
416 samples may have been grouped due to their location in the lower part of the catchment rather
417 than by their difference from the smaller streams, since they are distinguished by the presence

418 of the earlier mentioned phenols, which may come from tundra vegetation, and Cu, Ni and
419 Co, of which at least Cu should occur abundantly in the left tributaries of the lower part of the
420 catchment. The lake samples clustered around such characteristics as higher molecular weight
421 PAHs, some trace metals, HCHO and ACV, which indicates that in certain circumstances the
422 stagnant water may gain more toxic characteristics and be less habitable to the generalist
423 bacteria population, although the effect is not consistent across all samples.

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

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

443 4. Final remarks and conclusions

444 The Arctic environment, although it seems to be free from anthropogenic pollution, is not as
445 pristine as it might seem. Remarkable concentration levels of persistent organic pollutants
446 have been detected in the freshwater samples collected from the Revelva catchment. Globally
447 emitted contaminants accumulate in the Arctic and can be stored in this cold environment for
448 a long period of time (Mackay and Wania, 1995; Friedman and Selin, 2016). Moreover,
449 climate change influences the release of these contaminants through elevated melt rates,
450 resulting in increased contamination locally (Blais et al. 2001; Miner et al. 2018). The
451 microbial community interacts with contamination in the Arctic (e.g. Hauptmann et al. 2017),
452 however it is yet unknown how universal such interactions are. The important issue is to
453 know whether contaminants present in the environment are a toxic factor for bacteria, or
454 whether they show the ability to deal with these pollutants and reproduce in spite of their
455 presence. Described research shows that the catchment chosen for this study constitutes a
456 place of accumulation of persistent organic pollutants and also some trace elements that may
457 be toxic for the bacteria. The determined pollutants may pose a serious threat to the
458 development of bacteria in the Revelva catchment. Depending on their concentration in the
459 environment and simultaneous effects of their occurrence, they may or may not be an
460 effective inhibitor of bacterial growth in aquatic environments. However, despite the presence
461 of contaminants and the limited nutrient supply (described in Part 1, Kosek et al. submitted),
462 the Revelva catchment is characterised by a great biodiversity. The general structure of these
463 microbial communities was composed mainly of bacterial taxa, such as *Proteobacteria*,
464 *Actinobacteria*, *Bacteroidetes*, *Planctomycetes*, *Firmicutes* and *Verrucomicrobia*. The
465 bacterial ability to degrade toxic compounds depends on numerous factors, which were not
466 studied in this research, but there is a possibility that the bacteria present there decompose the
467 described contaminants. For example, *Bacteroidetes* are linked to the degradation of PAHs at

468 low temperature. Denitrifiers, as relatively abundant in this study *Flavobacterium*, sulphur
469 (*Desulfovibrio*, *Desulfotomaculum*, *Desulfosporosinus*) or psychrotolerant Fe-reducing
470 bacteria (*Rhodoferrax*) are also potentially capable of decomposing of the persistent organic
471 pollutants occurring in this catchment (Martin et al. 2012; Kappell et al. 2014). The potential
472 for biodegradation of polycyclic aromatic hydrocarbons (PAHs) at low temperature is not
473 well understood, but such biodegradation would be very useful for remediation of polluted
474 sites. Bacteria inhabiting the Revelva catchment have adapted to live in difficult conditions. It
475 can be hypothesised that they show the potential for decomposing persistent organic
476 pollutants, but in order to confirm it, it is necessary to conduct more thorough research at the
477 molecular level.

478 **Acknowledgments:** This work was supported by The National Science Centre, Poland,
479 research grant no. 2017/25/N/NZ9/01506. The authors would like to thank the Institute of
480 Geophysics PAS, and the staff of the Polish Polar Station Hornsund (especially D.
481 Dąbrowska) for supporting the field sampling.

482 **Conflict of Interests:** The authors declare no conflict of interest. The funding agencies had
483 no role in the design of the study; in the collection, analysis, or interpretation of data; in the
484 writing of the manuscript, and in the decision to publish the results.

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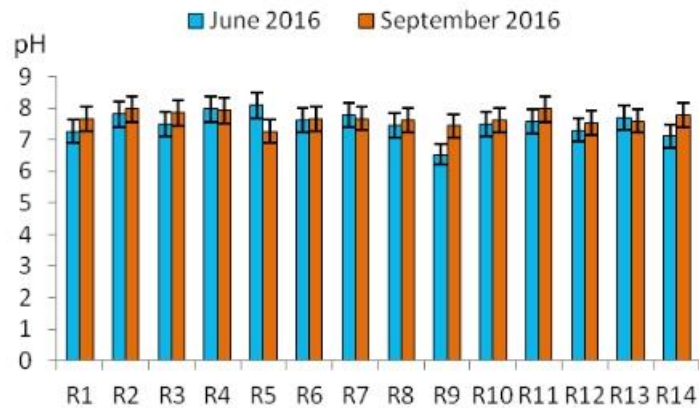
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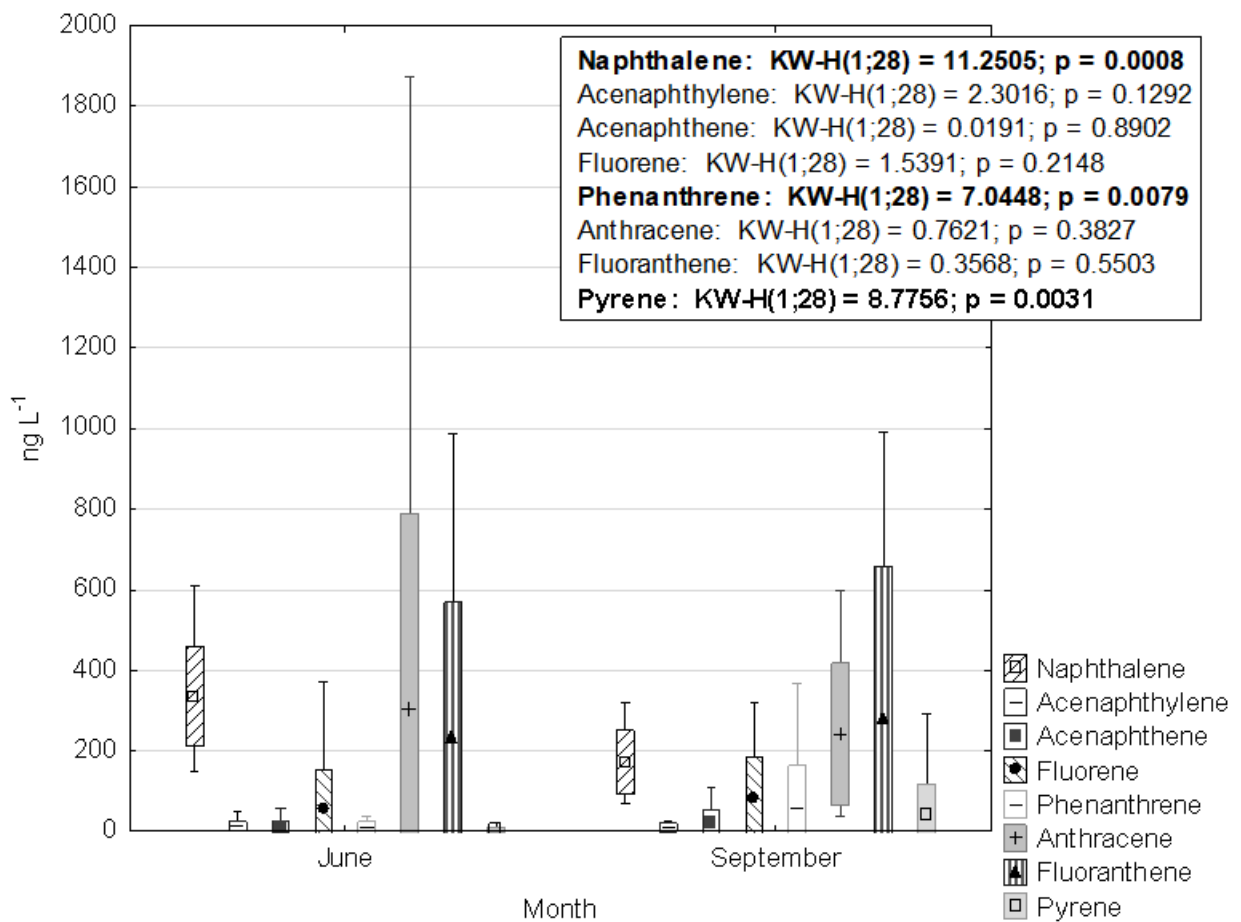
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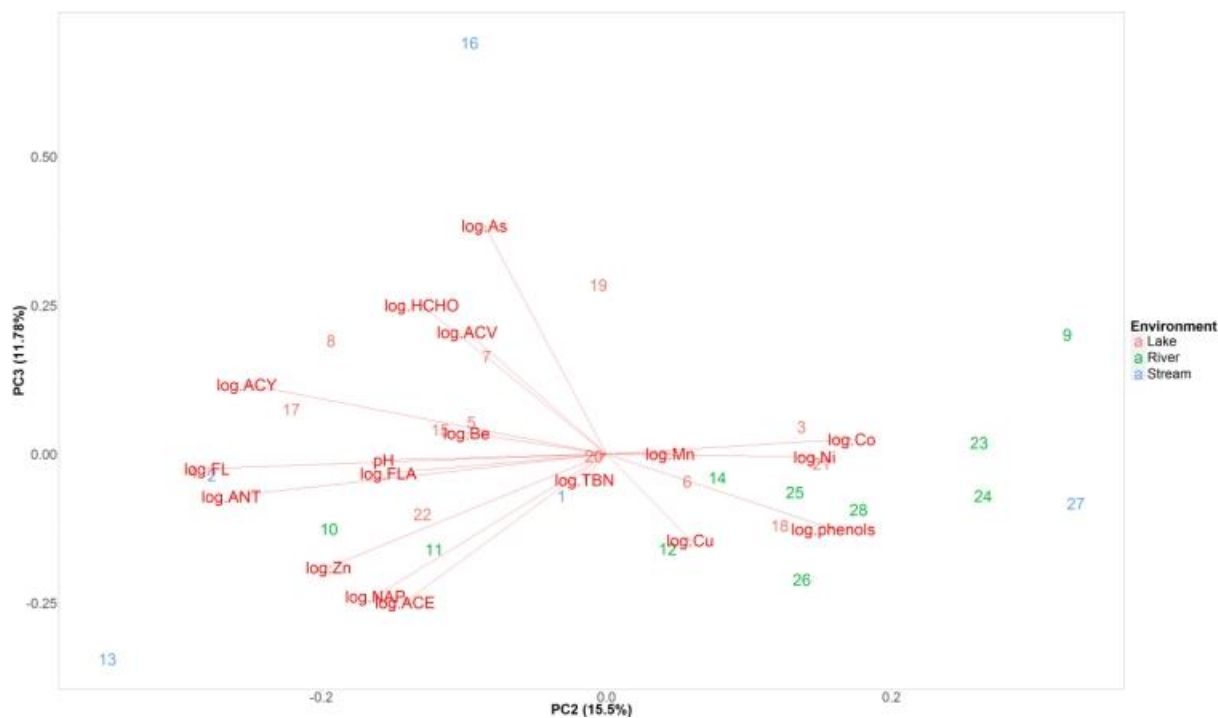
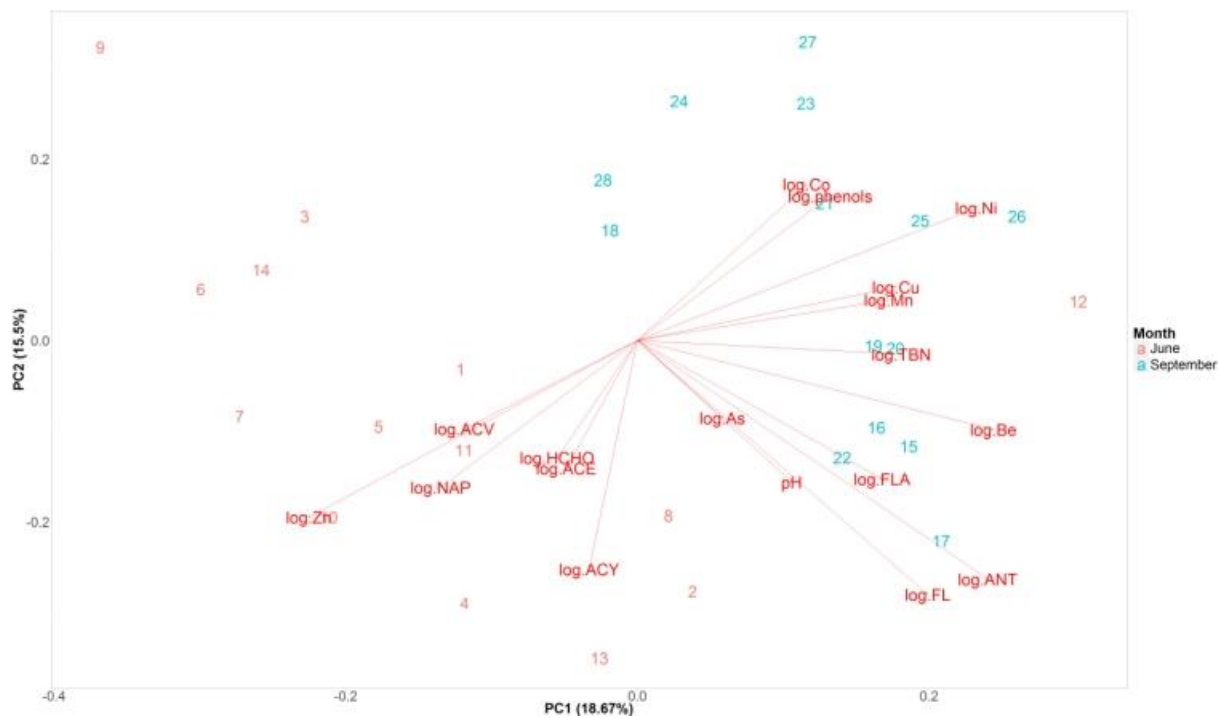
868 **Figure 1.** The pH values detected in freshwater samples collected in June and September

869 2016.

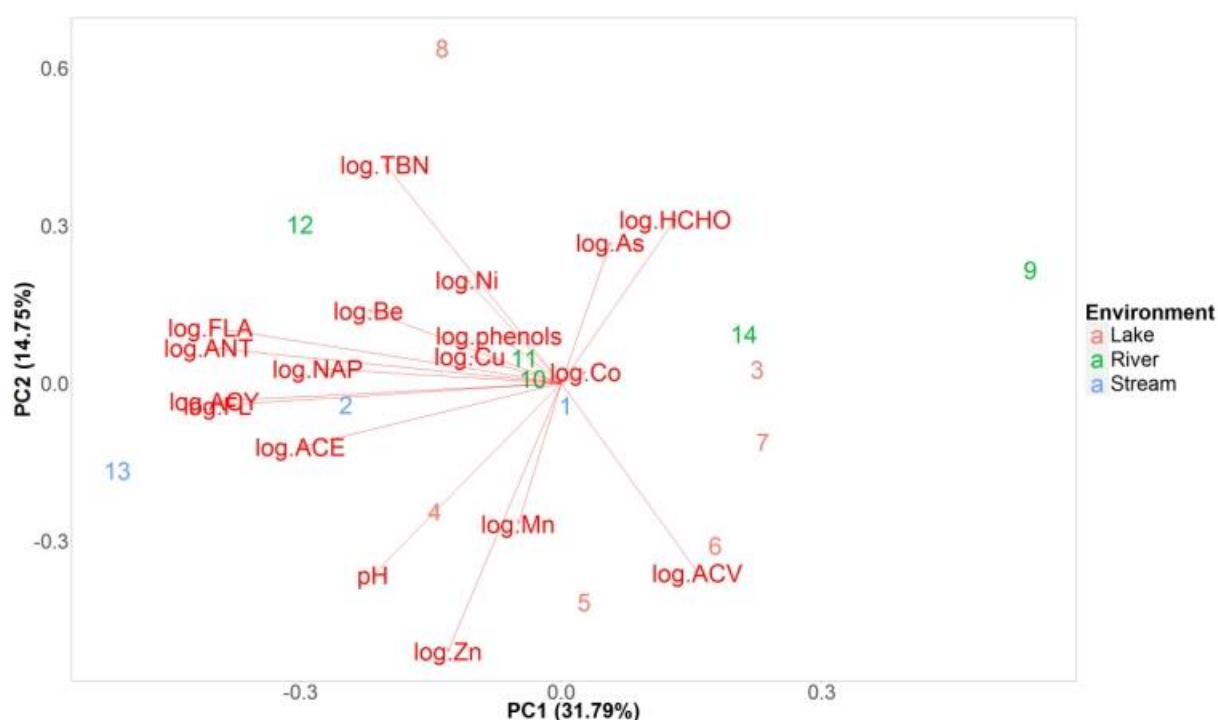


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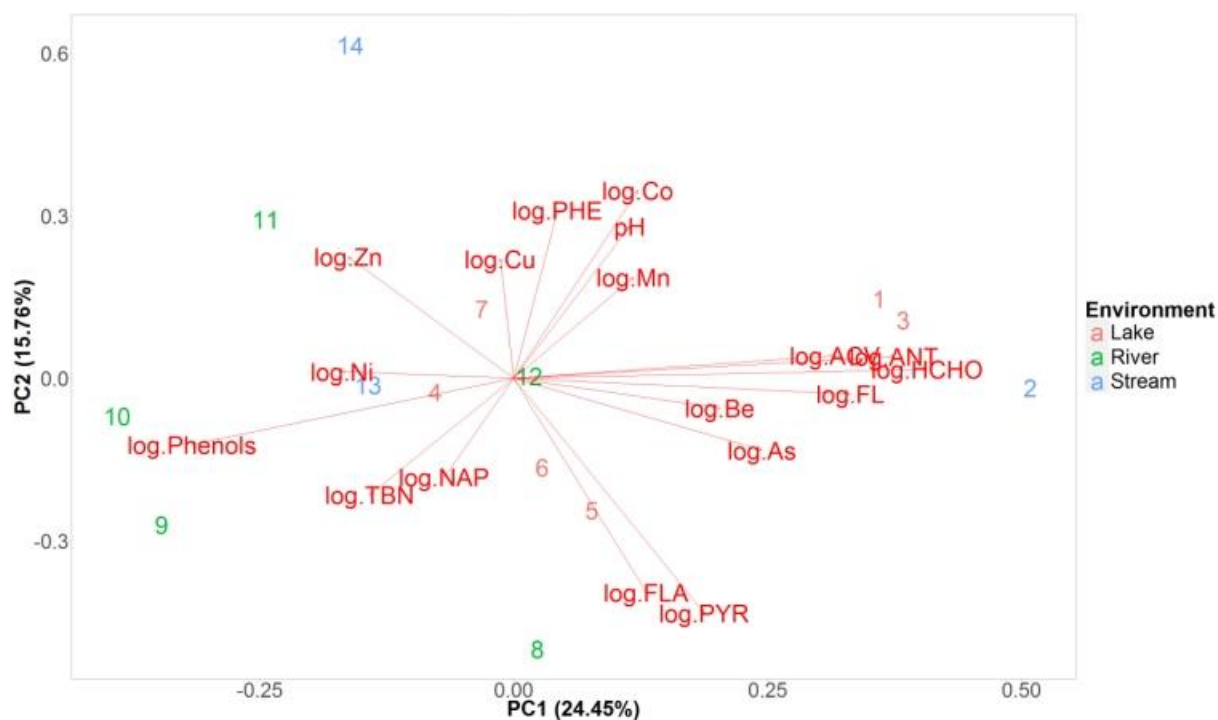
871 **Figure 2.** A box - whisker plot of the mean PAHs concentrations in water samples collected
 872 in June and September 2016. Significant differences between seasons are indicated with the
 873 non-parametric Kruskal-Wallis ANOVA p-levels below 0.05 and given in boldface. The box
 874 encompasses the mean value \pm SD, the whiskers show the full range of values noted (where
 875 $<$ LOD values were assigned a half of the LOD level).



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



885



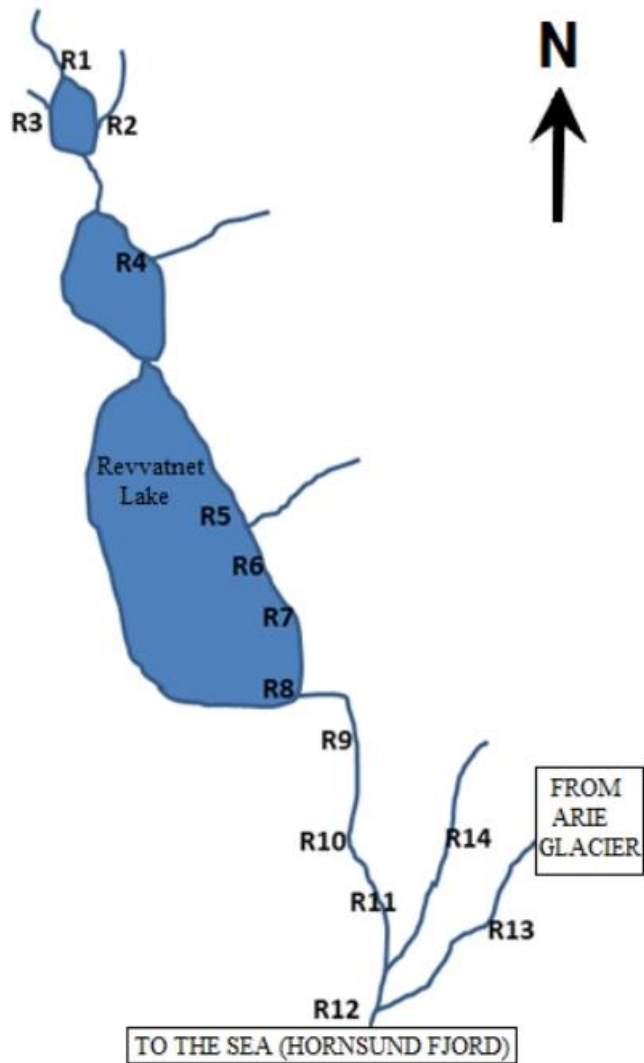
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887 **Figure 4.** PCA conducted for the potentially toxic chemicals detected in the different
 888 hydrological environments of the Revelva catchment (denoted by colour coding) and indices
 889 of the bacterial community structure in these waters.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

892

894 **Table 1.** Location of the sampling points in the Revvatnet catchment in Svalbard.

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



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

Determined compounds/parameters	Measurement range	LOD⁴	LOQ⁴	Measurement method/technique
pH	-	-	-	Electrochemical method: microcomputer pH-meter(Elmetron), electrode type EPS-1
∑ Phenols¹	0.025-5.00	0.001	0.003	Spectrophotometry method;
Formaldehyde¹	0.020-8.00	0.005	0.015	Spectrophotometer 6300, Jenway
PAHs²				Gas Chromatography technique coupled with Mass Spectrometry;
Naphthalene (NAP)	1.02-3500	0.034	1.02	Gas Chromatograph 7890A (Agilent Technologies) with the application of Mass Spectrometer (5975C inert MSD Agilent Technologies), detector (Agilent Technologies 5975C) with electron ionization
Acenaphthylene (ACY)	0.012-1000	0.004	0.012	
Acenaphthene (ACE)	0.012-1000	0.004	0.012	
Fluorene (FL)	0.005-1000	0.002	0.005	
Phenanthrene (PHE)	0.008-1000	0.003	0.008	
Anthracene (ANT)	0.023-1000	0.008	0.023	
Fluoranthene (FLA)	0.042-1000	0.014	0.042	



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

897 ¹[mg L⁻¹], ²[ng L⁻¹], ³[μg L⁻¹], ⁴the limit of detection (LOD) and the limit of quantification (LOQ) were calculated based on the standard deviation
898 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)

899

900 **Table 3.** Concentrations (\pm standard deviation, SD) of trace elements in the collected
 901 freshwater samples.

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

902
 903 **Table 4.** Concentrations (\pm standard deviation, SD) of PAHs, formaldehyde and the sum of
 904 phenols in the collected freshwater samples.

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

905

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

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



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



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

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

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