Heavy metal tolerance and polychlorinated biphenyl oxidation in bacterial communities inhabiting the Pasvik River and the Varanger Fjord area (Arctic Norway)

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26 **Abstract**

27 Heavy metals (HMs) and polychlorobiphenyls (PCBs) enter the Arctic environment through a
28 variety of anthropogenic sources with deleterious effects towards biota and public health.
29 Bacteria first transfer toxic compounds to higher trophic levels and, due to the tight link
30 existing between prokaryotic community functions and the type and concentration of
31 contaminants, they may be useful indicator of pollution events and potential toxicity to other
32 forms of life. The occurrence and abundance of HM-tolerant and PCB-oxidizing bacteria in
33 the sub-Arctic Pasvik river area, heavily impacted by anthropogenic modifications, was
34 related to HM and PCB contamination. This latter more likely derived from local inputs rather
35 than a global contamination with higher PCB and HM amounts (and higher bacterial viable
36 counts) that were determined in inner and middle sections of the River. Finally, a panel of
37 bacteria with potential applications in the bioremediation of cold environments were selected
38 and phylogenetically identified.

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41 **Key-words:** heavy metals, polychlorinated biphenyls, Arctic, multitolerance, *bph*A gene

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51 ## 1. Introduction

52 Freshwater environments represent zones strongly sensitive to pollution events (e.g. leak, spill
53 or other escape or deposit of a contaminant), since they tend to act as storage tanks for several
54 kinds of recalcitrant compounds, whose passage into the basin is driven by air (including
55 global atmospheric transport and precipitation) and/or direct discharges. Freshwater
56 catchments play a key role in the migration processes of pollutants from source to sink. In the
57 Arctic, pollutants deposited in the snow cover penetrate with percolating water to the soil and
58 groundwater and drain into surface waters, as well as contaminants deposited on the soil
59 surface migrate via drainage system into water systems (Kozak et al., 2016). For these
60 reasons, several Arctic freshwater catchments have been often analyzed for chemical
61 contamination to evaluate their influence on the water systems where they inflow (i.e. Nawrot
62 et al., 2016; Dauvalter and Kashulin, 2018; Kosek et al., 2018).

63 In polar areas a number of environmental and climatic features (e.g. seasonal daylight
64 conditions and extended periods of darkness during winter, ice coverage, low average annual
65 temperature, and snow precipitation) favour the potential accumulation of certain
66 contaminants in biotic and abiotic matrices (Hung et al., 2010). In particular, low temperature
67 is responsible for reducing both metabolic (which makes the excretion process less efficient)
68 and growth rates of organisms. Thus, the exposure of organisms to contaminants, through the
69 transfer along the food chain, has great environmental and human health concerns. The fate of
70 several environmental pollutants is strictly linked to bacteria, which represent the first step in
71 the transfer of toxic compounds to higher trophic levels (Gillan et al., 2005). Bacteria possess
72 genetic and biochemical capacities for remediation of HMs (e.g. by adsorbing, accumulating
73 and transforming them within food chains). Similarly, PCBs can be transformed, despite their
74 recalcitrant nature, into chemical substances by different microbial metabolic pathways, both
75 aerobic and anaerobic, facilitating further metabolization. Both HM-tolerance and PCB

76 oxidation have been reported for a number of different bacteria from cold environments
77 (Master and Mohn, 1998, 2001; De Domenico et al., 2004; Michaud et al., 2007; González-
78 Aravena et al., 2016; Lo Giudice et al., 2013; Neethu et al., 2015; Papale et al., 2017). The
79 interest in such bacteria resides in the fact that a tight link between prokaryotic community
80 functions (and composition) and the type and concentration of contaminants (e.g. HMs and
81 antibiotics) often exists, thus suggesting that microbes may be useful indicator of both
82 pollution events and potential toxicity to all other forms of life (Zakaria et al., 2004; Quero et
83 al., 2015; Laganà et al., 2018). Further, microbes with degradation/tolerance ability towards
84 contaminants under *in situ* environmental conditions could be exploited for the
85 bioremediation of contaminated cold areas.
86 In this context, the present study was aimed at establishing if the occurrence of HM-tolerant
87 and PCB-oxidizing bacteria in a sub-Arctic river, i.e. the Pasvik river, was related to HM and
88 PCB contamination. Inter- and intra-site variability of pollution and its impact on the bacterial
89 community was also evaluated to individuate locally-driven differences. Further, selected
90 bacterial isolates with biotechnological potential (i.e. tolerating high concentrations of HMs
91 or growing on a PCB mixture as sole carbon source) were further characterized and
92 phylogenetically identified.
93

94 **2. Materials and Methods**

95 *2.1. Sampling area*

96 The Pasvik River (sub-Arctic Norway), originating from the Lake Inari in Finland, is 166.6
97 km long, with a total catchment of 20,890 km^2, of which 69.8% belongs to Finland, 25.2% to
98 Russia and 5% to Norway. Fluctuations in the water level is low, less than 80 cm, with
99 periods of absence of ice between May/June and October/November. The average
100 temperature is around 12°C and maximum temperatures are registered during the summer

101 (17-18°C). The Pasvik River is heavily impacted by several anthropogenic sources, mainly
102 caused by emissions from smelters, metallurgic and mining activities, and domestic sewage
103 from the surrounding areas (Dauvalter and Kashulin, 2018). The Pechenganickel Company
104 represents the main local source of contamination for the area and, for such reason, regular
105 monitoring of both sediment and water have been performed during last decades to evaluate
106 the environmental status of the Pasvik river (Ylikörkkö et al., 2014). The Pasvik system is
107 constituted by several lakes and reservoirs, mires, linked by short sections of river, and its
108 outlet is located in the Kirkenes area that therefore results enriched with pollutants (Dauvalter
109 and Rognerud, 2001). It is argued that the river flow could have a pollutant effect also on the
110 fjord system up to the larger Varanger fjord.
111
112 *2.2. Collection and preliminary treatment of samples*
113 In the framework of the SedMicro project, water and sediment samples were collected from
114 ten stations along the Pasvik River and the Varanger Fjord (Arctic Norway) area between 17
115 and 24 July, 2013 (Figure 1). Based on their location, stations were subdivided in three
116 groups, as follows: *outer* (stations 1 and 17), *middle* (stations 9, 18 and 19) and *inner* (stations
117 5, 8, 13, 15 and 16) stations. All samples for microbiological analyses were aseptically
118 collected by using pre-sterilized 1-L polycarbonate bottles (water) or containers (sediment),
119 except for three samples (sampling sites 17, 18 and 19) that were collected by scuba. Samples
120 were preliminary processed after sampling (approximately 2 h) in the laboratory of the NIBIO
121 Svanhovd Research Station (Svanvik, Pasvik Valley), as described in the following sections.
122 Sediment samples for chemical analyses were collected using a pre-cleaned stainless steel
123 bailer and stored at -20°C in pre-cleaned glass jars until analysis. Samples were named using
124 the numbers 1 to 19 followed by the suffix S (for sediment samples) or W (for water
125 samples).

126 Salinity, pH, and temperature of water and sediment were recorded at each sampling point
127 (Table 1).
128
129 *2.3. Chemical analyses of sediment samples*
130 2.3.1. Heavy metal concentration
131 All solutions were prepared using LC-MS Ultra Chromasolv water (Sigma Aldrich) in a class
132 100 laboratory dotted with a workbench with vertical airflow. Nitric acid (67-69 %
133 Suprapur®, Romil) and H_2O_2 (30 % Suprapur®, Romil) were applied. All the material was
134 soaked in HNO_3 10% for at least 16 h, and then rinsed with double distilled water.
135 Each sediment sample aliquot (about 0.2 g) was weighed using an analytical balance (AE 200
136 Mettler) in a Quartz container and then placed in an HPR 1000/6 rotor. Prior to their use, the
137 inner liners were soaked in 10% HNO_3 overnight. The following microwave cleaning
138 procedure was carried out: 5 mL of 67-69% HNO_3 was added to each liner, the digestion
139 vessels were sealed and the temperature was raised to 180°C within 10 min and held at 180°C
140 for 10 min. After cooling, the contents of the vessels were discarded and the liners were
141 thoroughly rinsed with double distilled water. Ten milliliters of 67-69% nitric acid and 3 ml
142 of 30% hydrogen peroxide were added to the samples, submitted to microwave assisted
143 mineralization in a high performance microwave digestion unit, MLS 1200 mega (Milestone,
144 Brøndby, Denmark) with an MLS Mega 240 terminal and EM 45 A exhaust module.
145 The following optimized microwave program was implemented: 250 W: 5 min; 400 W: 6
146 min; 0 W: 5 min; 500 W: 2 min; 300 W: 3 min; ventilation: 8 min. The program used was
147 based on the manufacturer's recommendations for use (Milestone Cookbook of Microwave
148 Application Notes for MDR technology, January 1995). The rotor was then cooled down for a
149 period of 1 h. Mineralized solutions were quantitatively transferred into 50 mL volumetric
150 flasks and diluted to volume with ultrapure water. Quartz containers were rinsed at least 3

151 times with ultrapure water to dilute any possible rest of colloids attached in the vessels' walls.
152 Triplicate digested reagent blank solutions for each digestion run were analyzed for
153 determination of the method detection limit (MDL).
154 A quadrupole ICP-MS Agilent model 7700 (Agilent Technologies, Tokyo, Japan) equipped
155 with a collision cell system for elimination of isobaric interferences was used for sediment
156 sample analysis (Table S1). Samples were introduced into the plasma by an autosampler
157 Agilent model ASX-520 (Agilent Technologies, Tokyo, Japan). The instrument was fitted
158 with a MicroMist nebulizer (Agilent nebulizer standard for 7700) with a Scott-type double-
159 pass glass spray chamber cooled down to 2°C.
160 The external calibration solutions must include known concentrations of each target analyte.
161 They were prepared from standard certified elemental solutions (ICUS-1239; Sb, As, Ba, Be,
162 Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Se, Tl, Sn, Zn, V; 100 µg/mL) and LC-MS Ultra
163 Chromasolv water containing 2% HNO_3 to get a range of concentrations: 1.0, 2.0, 5.0, 10.0,
164 20.0, 50.0, 100.0, 200.0 and 500 μg/L. A blank solution consisting in LC-MS Ultra
165 Chromasolv water containing 2% HNO_3 completed the calibration curve (counts versus μg/
166 L) for each analyte.
167 The nonspectral matrix effects associated to the ICP-MS measurements were resolved by the
168 addition of internal standards. The standard solution was prepared by diluting a single
169 elemental stock solution with LC-MS Ultra Chromasolv water containing 2 % HNO_3 up to get
170 10 μg/L of iridium (^{193}Ir).
171 Each individual sample was measured with three main acquisition runs during the experiment,
172 providing mean values and standard deviation. The average recovery of the internal standard
173 (iridium) was 100 ± 5%, indicating the absence of a significative matrix effect in the adopted
174 procedure. Since some metal concentrations are near the detection limits of ICP-MS, a
175 validation of the analytical technique was mandatory. To evaluate the accuracy of the

176 instrument itself, marine sediment sample certified for heavy metals MURST-ISS-A1
177 (Antarctic Marine Sediment, Programma Nazionale Ricerca Antartide, Istituto Superiore
178 Sanità) was analyzed (Table S2). The limit of detection (LOD) for this study, based on the
179 mean of the blank samples from all runs plus three times its standard deviation, were obtained
180 within the range of 0.002 μg/g (Cd) to 0.1 μg/g (Mn) for sediment samples. Sample blanks
181 and standard solutions were run with each batch of samples as quality control. Digestion and
182 ICP-MS analysis were repeated three times for 6 samples, to estimate the reproducibility of
183 the entire procedure. The maximum relative standard deviation obtained was 11%.
184

185 2.3.2. Polychlorinated biphenyl concentration

186 Chemical analyses to determine the PCB concentration in sediment samples were carried out
187 as previously reported (Papale et al., 2017). Briefly, sediment extraction was carried out
188 according to Jensen et al. (1977; EPA Method 3660 B: Sulfur Cleanup). An aliquot of the wet
189 sediment sample (2-3 g) was dried at 110°C for sediment dry weight estimation. Sediment
190 (15-20 mg) was transferred into a weighed centrifuge tube (60 mL) and centrifuged for 15
191 min at 2500 x g (4800 rpm; Rotofix 32 A, Hettich). The water was discarded, the tube
192 weighed again, and the wet weight calculated. 20 mL of a mixture of acetone: hexane (3:1)
193 was added to the sample and this was posed in a ultrasonic bath (Sonorex super 10 p,
194 Bandelin) at 60°C for 30 min. The supernatant was taken and the sediment was resubmitted to
195 the same treatment. The two organic phases were then combined and placed in a centrifugal
196 evaporator (Jouan RC10.22 series Vacuum Concentrator). After a first concentration the
197 residue was dissolved in trimethyl pentane (4 ml) and again placed in the centrifugal
198 evaporator to obtain a final volume of 2 mL. The trimethyl pentane extract (2 ml) was shaken
199 with 2-propanol (1 mL) and the TBA-sulfite reagent (solution of
200 tetrabutylammoniumhydrogen sulfate saturated with sodium sulfite, Sigma-Aldrich) (1 mL)

201 for 1 min. If the sodium sulfite precipitate disappeared, more was added in 100 mg portions
202 until a solid residue remained after repeated shaking. Water (5 mL) was added and the tube
203 was shaken for another minute, followed by centrifugation, and the trimethyl- pentane phase
204 was transferred to a test tube. In accord with the method EPA 3620C to separate analytes
205 from non-polar or slightly polar interfering compounds was used a chromatographic column
206 containing the adsorbing solid phase (Florisil) with a top layer of sodium sulfate, to remove
207 any traces of water (Dual Layer Florisil®/Na_2SO_4 SPE Tube 2g/2g/6mL Fluorisil particles
208 diameter 150/200 µm, Supelco). At the end of the purification samples were concentrated
209 using the centrifugal evaporator to a final volume of about 1 mL, checked by weighing. To
210 each sample 10 µL of an injection standard (MBP-CP solution/mixture of PCB77, PCB81,
211 PCB126 and PCB169, final concentration 5 ng/mL; Wellington Laboratories) was added
212 before being injected into the gas chromatograph. A gas chromatograph mod. 5890A
213 (Agilent, USA), equipped with a PTV injection system mod. 6890, and coupled with a mass
214 spectrometric detector mod. 5973A (Agilent, USA) operating both in SIM (Selected Ion
215 Monitoring) and TIC (Total Ion Current) modes, was used for the determination of PAHs. In
216 both cases, chromatographic separation was performed on a fused silica capillary column MS-
217 5 (Hewlett Packard Italiana) 95% dimethyl–5% phenyl polysiloxane chemically bonded
218 stationary phase, 0.25 mm internal diameter, 0.25 µm film thickness, 30 m length; the column
219 was connected to a 5 m long 0.32 mm internal diameter deactivated fused silica capillary pre-
220 column. The temperature profile of the chromatographic oven was the following: initial
221 temperature, 50°C, isothermal for 5 min; 15°C/min up to 150°C and isothermal for 1 min,
222 5°C/min up to 240°C, 25°C/min up to 300°C and isothermal for 5 min. In order to perform
223 large sample volume injections, the injection system, equipped with a deactivated glass wool
224 packed insert and with the split valve open, was kept at 55°C during the initial 30 s, then the
225 split valve was closed and the injector heated at 500°C/min up to 280°C. Helium 99.995%

226 purity (Rivoira, Italy) was used as a carrier gas at constant flow (1 mL/min) and 90 kPa at
227 initial temperature. The sample volume injected was 40 μL, unless otherwise specified. Each
228 individual sample was measured with three main acquisition runs during the experiment,
229 providing mean values and standard deviation.

230

231 *2.4. Estimation of prokaryotic cell abundance in natural samples*

232 2.4.1. Total prokaryotic cell abundance

233 Samples for total prokaryotic cell abundance (TCs) estimation were fixed with formaldehyde
234 (final concentration, 2%), filtered on black polycarbonate membranes (pore size, 0.22 μm;
235 diameter 25 mm) and kept frozen until processing. Sediment samples were preliminarily
236 subjected to a cell detachment treatment (Griebler et al., 2001). DAPI (4',6–diamidino–2–
237 phenilindole)-stained cells were counted by a Zeiss AXIOPLAN 2 *Imaging* epifluorescence
238 microscope as previously described (Porter and Feig, 1980; La Ferla et al., 2004). More than
239 300 cells per sample were counted in randomly selected fields.

240

241 2.4.2. Viable counts

242 For viable counts (VCs) Serial dilutions were prepared (1:10 and 1:100, using 0.9% sodium
243 chloride solution) and 100 μL of each dilution was spread-plated in duplicate on Plate Count
244 Agar (PCA; Difco). Inoculated plates were incubated in the dark at 4°C for one month.
245 Results were expressed as CFU/mL and CFU/g for water and sediment, respectively.

246

247 *2.5. Bacterial tolerance to HMs*

248 2.5.1. Estimation of viable counts on media amended with HMs

249 VCs in the presence of heavy metal tolerance were estimated on PCA amended with heavy
250 metal salts. Metal salt solutions were prepared in filter-sterilized phosphate buffer saline

251 (PBS) and stored at 4°C until use (Selvin et al., 2009). Individual heavy metal salts (i.e. $CdCl_2$
252 · 2.5 H_2O, $HgCl_2$, $CuCl_2$ · $2H_2O$ and $ZnCl_2$) were added at four different concentrations: 50,
253 500, 1000 and 5000 ppm. Aliquots (100 μL) of each sample were spread plated in duplicate
254 and incubated on 4°C for 1 month under aerobic conditions. Results were expressed as
255 Colony Forming Units per mL (CFU/mL) and gram (CFU/g) for water and wet sediment,
256 respectively.
257
258 2.5.2. Isolation of HM tolerant bacteria
259 Colonies were randomly isolated from agar plates amended with heavy metal salts, picked
260 and subcultured almost three times under the same conditions before being considered pure.
261 Cultures were routinely incubated in the dark at 4°C on PCA.
262
263 2.5.3. Screening for HM multi-tolerance
264 Each isolate was assayed for tolerance to heavy metals different from that used in the
265 isolation medium. Metal salt solutions were prepared and added to the medium as described
266 above for viable counts. Isolates were streaked on HM-amended media and plates were
267 incubated at 4°C for 1 month under aerobic conditions.
268
269 *2.6. Selection of PCB-oxidizing bacterial isolates*
270 2.6.1. Estimation of viable counts on media amended with biphenyl
271 VCs in the presence of biphenyl (BP) were estimated on solidified (1.5% agar, w/v) Bushnell
272 Haas (BH; Difco) by plating aliquots (100 μL) of the natural samples. BP was added as
273 crystals in the Petri dish lid after inoculation. Replicate plates were incubated at 4°C for 30
274 days. Results were expressed as CFU/mL and CFU/g for water or wet sediment, respectively.

275

276 2.6.2. Set-up of bacterial enrichment cultures from sediment samples

277 For the initial enrichment, biphenyl (BP) served as the sole carbon and energy source for
278 growth, as follows. Aliquots (1 mL) of a stock solution (75 mg/mL) of BP dissolved in
279 chloroform was added to empty Erlenmeyer flasks and the solvent was allowed to evaporate.
280 Then, 10 g of wet sediment samples were used to inoculate 75 mL of filter-sterilized seawater
281 (collected from the same site at sampling time) in BP containing flasks (final concentration
282 0.1%, w/v). An additional culture flask was set up for each sample with no added carbon
283 source to serve as a negative control. Two successive enrichments were prepared in Bushnell
284 Haas (BH; Difco) minimal medium by using 10% (w/v) culture fluid used as the inoculum for
285 each subculture at 3-week intervals. All cultures were incubated aerobically at 4°C with
286 shaking at 175 rpm for 1 month. Results were expressed as CFU/g of sediment.

287

288 2.6.3. Bacterial isolation

289 Aliquots (100 µL) of the final enrichment were plated on solidified BH. BP was added as
290 crystals in the Petri dish lid after inoculation. Replicate plates were incubated at 4°C for 30
291 days. BH *plus* NaCl agar plates without BP were used as a control. For bacterial isolation,
292 colonies were randomly selected from agar plates, picked and subcultured three times under
293 the same conditions.

294

295 2.6.4. Bacterial growth in the presence of Aroclor 1242

296 Bacterial growth in the presence of PCBs was tested in liquid BH as previously reported
297 (Michaud et al., 2007). Aroclor 1242 (Sigma-Aldrich; 100 ppm in dichloromethane) was
298 added as sole carbon and energy source (final concentration 0.1%, w/v). Aroclor 1242 is a
299 mixture of PCB congeners (ranging from dichloro- to hexachlorobiphenyls) made of twelve

300 carbon atoms in the biphenyl molecule and containing 42% chlorine by weight (Frame et al., 301 1996). Cultures were incubated at 4°C for one month. The ability to use PCBs as growth 302 substrates was evaluated according to the degree of turbidity or the appearance of cellular 303 flocs in the test tubes. Uninoculated medium was incubated in parallel as a negative control.

304

305 2.6.5. Screening for the *bph*A gene

306 Strains showing the ability to grow in the presence of Aroclor 1242 as the sole carbon and 307 energy source were screened for the presence of the catabolic gene *bph*A involved in PCB 308 degradation. The presence of genes was determined by PCR, using specific primers: 309 2BPHFWD1 (5' ADVCCSCGBGCCGCBTCHTCG 3') and 2BPHREV1 (5' 310 ADVCCSCGBGCCGCBTCHTCG 3') (Master and Mohn, 2001). The reaction mixture was 311 assembled at 0°C and contained 1 µL DNA, 1 µL of each of the two primers (10 µM), 0.4 µL 312 of each dNTP (10 mM), 2 µL of reaction buffer 10X, 0.4 µL of BSA (2.5%), 0.4 µL of *Taq* 313 polymerase 5 PRIME (5U/µL), and sterile Milli-Q water to a final volume of 20 µL. Negative 314 controls for DNA extraction and PCR setup (reaction mixture without a DNA template) were 315 also used in every PCR run, DNA from *Burkholderia xenovorans* (DSM17367) was used as 316 positive control. The PCR program was as follows: 1) 95°C for 5 min; 2) 35 cycles at 94°C 317 for 45 s, 58°C for 1 min and 72°C for 2 min; 3) 72°C for 10 min (Lehtinen et al., 2013). The 318 results of the amplification reactions were analyzed by agarose gel electrophoresis (2%, w/v) 319 in TAE buffer (0.04 M Tris-acetate, 0.02 M acetic acid, 0.001 M EDTA), containing 1 µg/mL 320 of ethidium bromide and the fragment size was evaluated through the use of a 1 kb ladder 321 (Fermentas).

322

323 *2.7. Phylogenetic affiliation of bacterial isolates*

324 2.7.1. PCR amplification, sequencing and analysis of 16S rRNA gene from bacterial isolates

325 Single colonies of selected strains were lysed by heating at 95°C for 10 min. Amplification of
326 16S rRNA gene was performed with a ABI 9600 thermocycler (PE, Applied Biosystems) by
327 using Bacteria-specific primers 27F (5' – AGA GTT TGA TC(AC) TGG CTC AG – 3') and
328 1492R (5'-CTACGGCTACCTTGTTACGA-3') (Rizzo et al., 2013). The reaction mixtures
329 were assembled at 0°C and contained 1-10 ng DNA, 10X buffer, 3 mg/mL BSA, 10 µM of
330 each forward and reverse primer, 10 mM dNTP, 5 U/µL of 5Prime polymerase and sterile
331 distilled water to a final volume of 20 µL. The PCR program was as follows: 1,30 min at
332 95°C, followed by 5 cycles of 30 sec at 95°C, 30 sec at 60°C, 4 min at 72°C; 5 cycles of 30
333 sec at 95°C, 30 sec at 55°C, 4 min at 72°C; 25 cycles of 30 sec at 95°C, 30 sec at 50°C, 4 min
334 at 72°C and a final extension step of 10 min at 60°C. The expected size of the PCR product
335 was approximately 1.4 kb. The results of the amplification reactions were analyzed by
336 agarose gel electrophoresis (0.8%, w/v) in TAE buffer (0.04 M Tris-acetate, 0.02 M acetic
337 acid, 0.001 M EDTA), containing 1µg/mL of ethidium bromide.
338 Sequencing was carried out at the Macrogen Europe (Netherlands). Next relatives of isolates
339 were determined by comparison to 16S rRNA gene sequences in the NCBI GenBank and the
340 EMBL databases using BLAST, and the "Seqmatch" and "Classifier" programs of the
341 Ribosomal Database Project II (http://rdp.cme.msu.edu/) (Altschul et al., 1997). Sequences
342 were further aligned using the program Clustal W (Thompson et al., 1994) to the most similar
343 orthologous sequences retrieved from database. Each alignment was checked manually,
344 corrected and then analyzed using the Neighbour-Joining method (Saitou and Nei, 1987)
345 according to the model of Jukes-Cantor distances. Sequences with a ≥97% similarity were
346 grouped in the same operational taxonomic unit (OTU).
347
348 2.7.2. Nucleotide sequence accession numbers

349 The nucleotide sequences of the selected bacterial isolates have been deposited in the
350 GenBank database under the following accession numbers: **MH231459**-**MH231491** (HM-
351 tolerant isolates) and **MH244129**-**MH244157** (PCB-oxidising isolates).

352

353 *2.8. Statistical analysis*

354 Results were analyzed by correlation coefficient to measure the relationship eventually
355 existing between microbial counts and pollutant concentrations. All data were elaborated by
356 performing one-way ANOVA and post-hoc analysis (Tukey test) to compare stations and
357 detect significant differences in relation to pollutant concentration and microbial abundances
358 (MiniTab software, version 16.0). Results were considered significant when $p \leq 0.05$.
359 In addition, non-metric multidimensional scaling (nMDS) was performed to cluster the
360 stations in relation to heavy metal and PCB concentrations, and to verify the potential
361 influence of station location. Principal Component Analysis (PCA) was computed to identify
362 the potential relationship between chemical and biological parameters at each station (Primer
363 6 Plymouth Marine Laboratory, Roborough, United Kingdom).

364

365 **3. Results**

366 *3.1. Chemical analyses of sediments*

367 3.1.1. HM concentration

368 Results on the HM concentrations in sediment samples are reported in Table 2. The highest
369 concentrations were detected for Fe and Mg, with maximum levels for Fe at stations 1S and
370 8S (6189.2 and 5830.3 ppb, respectively), and Mg at stations 1S and 19S (4076.4 and 10825.5
371 ppb, respectively). Among the inner stations, the level of Fe was in the order 16S<5S<8S,
372 with a low decrease at middle stations until an increase at outer stations, in the order 17S<1S.

With regard to Mg, its levels were in the order 15S<5S<8S at inner stations, with an increase at middle stations (9S<19S) and then a decrease at the inner station 1S.

Globally, the stations 1S (among outer stations), 9S (among middle stations) and 5S and 8S (among inner stations) resulted particularly polluted if compared to the other stations analyzed in this study . In addition to Fe and Mg, the stations resulted primarily polluted by Mn and Zn, followed by Ni and Cu. Remarkable amounts of Mn were detected at stations 1S (i.e. 131.4 ppb), 8S (i.e. 71.2 ppb) and 5S (i.e. 72.6 ppb), whereas Zn resulted particularly abundant at stations 13S (i.e. 89.6 ppb), 8S (i.e. 82.4 ppb) and 17S (i.e. 61.2 ppb). The station 8S appeared to be strongly polluted also by Ba, with a concentration of 118.1 ppb. Zn levels varied among the stations with a gradual decrease from inner to outer stations (in the order 13S<5S in the outer, 19S<9S in the middle and 1S<17S in the outer areas), while Mn levels showed a weak increase (in the order 15S<8S<5S in the inner area). Low amounts of Be, As, Se, Mo, Cd, Sn, Sb, Hg and Tl were detected at all stations. Pb was detected at high concentrations at station 13S (27.4 ppb). The one-way ANOVA showed no significant differences among stations in relation to HM concentrations ($p > 0.05$). Otherwise, significant differences were detected among HM concentrations, since Fe and Mg amounts were significantly higher ($p < 0.05$).

All HM amounts in sediment samples were divided for area (inner, middle and outer) and analyzed by both one-way ANOVA and Tukey test to detect potential significant differences among areas. All HM amounts resulted comparable at inner, middle and outer stations.

The nMDS analysis was computed on HM concentrations among stations considering the station location (i.e. outer, middle, inner) as a factor. Stations clusterized in four main groups: *SEDa*, including stations 15S, 16S, 17S, 18S, 5S and 19S; *SEDb* including stations 1S and 8S; *SEDc* and *SEDd* including stations 9S and 13S, respectively (Figure 2a). The ANOSIM test, computed with above definite clusters as factors, revealed a significant difference (Global $R = 0.9$, $p = 0.002$) among the groups, with higher significant difference between *SEDa* and *SEDb*,

398 as resulted from the pairwise Tests. An additional ANOSIM test, computed with station
399 location as factors showed that no significant differences occurred.
400
401 3.1.2. PCB concentration
402 A total of 22 PCB congeners were detected at concentrations that ranged between 0.01 and
403 126.4 ng/g (Table 3). PCB018, PCB029, PCB050, PCB104 and PCB201 did not occurred in
404 analyzed samples. Stations 1S, 9S, 16S and 13S resulted the most polluted by PCBs. High
405 concentrations of PCB008 (2,4'-dichlorobiphenyl) were detected in almost all stations, with
406 the only exception of stations 15S and 13S. The maximum amount of PCB008 was recorded
407 at the station 9S (126.64 ng/g), followed by the station 6S (24.62 ng/g). This station resulted
408 polluted also by PCB028 (2,4,4'-trichlorobiphenyl) and PCB187 (2,2',3,4',5,5',6-
409 heptachlorobiphenyl) with concentrations of 15.41 and 14.22 ng/g, respectively. The one-way
410 ANOVA showed that significative differences occurred among stations in relation to PCB
411 concentration (p= 0.03). Indeed, station 9S was characterized by significantly higher values
412 than other stations, even if the Tukey test showed less marked differences with stations 9S,
413 16S and 13S. The PCB concentration among the sampling sites highlighted a significative
414 difference only for PCB008, whose amount was strongly higher than all the other PCB
415 congeners (p= 0.03). These results are confirmed by the nMDS analysis computed on PCB
416 concentrations (Figure 2b), with an evident separation of stations 9S and 13S from the others.
417 All PCB concentrations were also analyzed in function of the sampling area (i.e. inner, middle
418 and outer) by one-way ANOVA and tested by using the Tukey test. No significant differences
419 occurred among PCB levels at inner, middle and outer stations.
420
421 *3.2. Prokaryotic abundance*
422 3.2.1. Total and viable counts

423 Results on TCs and VCs are reported in Table 4. TCs in sediment were three orders of
424 magnitude higher than in water. Briefly, TCs in water samples ranged from 0.2 ± 0.4 x 10^6
425 (station 17W) to 6.3 ± 0.7 x 10^6 cells/mL (station 8W), whereas they were between 1.2 ± 0.4 x
426 10^9 (station 16S) and 20.3 ± 8.5 x 10^9 cells/g (station 18S) in wet sediment.
427 VCs on PCA in water samples were between 1.6 ± 0.8 x 10^2 (station 1W) and 16.0 ± 0.8 x 10^2
428 CFU/mL (station 19W). They resulted uncountable due to a massive growth at several
429 stations (referred to *nd* in Table 4). VCs in sediment samples resulted one order of magnitude
430 higher than in water samples, ranging from 1.0 ± 0.1 x 10^3 (station 1S) and 1070 ± 114 x 10^3
431 CFU/g (station 18S) of wet sediment.
432 A positive correlation was detected by statistical analysis between TCs and VCs, both for
433 water and sediment samples.
434 VCs on BP-amended plates in sediment samples were between 0.2 ± 0.1 (station 1S) and 53.8
435 ± 4.4 x 10^2 CFU/mL (station 18S). They resulted uncountable due to a massive growth at
436 three stations (referred to *nd* in Table 4). VCs on BP-amended plates in water samples were
437 between 0.9 ± 0.5 (station 5W) and 28.8 ± 3.1 x 10^2 CFU/mL (station 9W). A significant
438 difference was detected by one-way ANOVA between VCs obtained from BP-amended plates
439 of water and sediment samples ($p<0.05$), with values significantly higher for sediment
440 samples.
441
442 *3.3. Heavy metal tolerance*
443 3.3.1. Estimation of viable counts on media amended with heavy metals
444 Overall, tolerance of HMs in water and sediment samples was in the order Cu>Zn>Cd>Hg. In
445 water samples bacterial growth occurred up to 50 ppm of Cd and Hg, and up to 1000 ppm of
446 Zn and Cu. No growth occurred at the highest (5000 ppm) concentration tested. The same was
447 true for sediment samples, with the exception of Cu that was tolerated up to 500 ppm (Table

448 4). With regard to water samples, the highest values of VCs were determined in the presence
449 of Zn and Cu at stations 17W (50 ppm of Zn: 14.6 ± 0.3 x 10^2 CFU/mL) and 16W (500 ppm
450 of Cu: 41.6 ± 0.8 x 10^2 CFU/mL), respectively. VCs in sediment samples were two orders of
451 magnitude higher than water samples. As for water, in sediment the highest values were
452 determined for Zn up to 1000 ppm (between 17.5 and 2.15 CFU/g; stations 1S and 19S) and
453 Cu (up to 158.0 ± 22.3 x 10^4 CFU/g; station 17S).
454 Differences in tolerance patterns were observed among stations with bacterial communities
455 that generally tolerated up to three HMs in water and a single HM in sediment (Table 4). The
456 bacterial communities (in both sediment and water) from station 19 were the sole to tolerate
457 Hg, thus showing tolerance to the four HMs tested. Conversely, no growth occurred at
458 stations 8W, 15S and 16S in the presence of metals. In water samples, Cu was generally
459 tolerated up to 500 ppm. Exceptions were stations 9W and 16W as bacterial growth was
460 observed up to 1000 ppm of this metal. Zn, Hg and Cd were generally tolerated up to 50 ppm.
461 Exceptions were three stations where Zn was tolerated up to 500 ppm (stations 9W and 15W)
462 or 1000 ppm (station 16W). In sediment samples, the bacterial communities generally
463 tolerated up to 50 ppm of each tested metal. However, Cu was also tolerated up to 500 ppm at
464 stations 8S, 17S and 19S. Bacterial growth in the presence of Zn was observed at four
465 stations, including the station 1S where such metal was tolerated up to 1000 ppm. Finally, Cd
466 was tolerated only at stations 8S and 19S.
467 With regard to viable counts on HM-amended plates (final concentration 50 ppm) no
468 significative differences were detected among water and sediment samples. When considering
469 only the sediment samples, the abundances detected at the station 19S resulted significantly
470 higher than at the other stations ($p= 0.03$).
471 All data were also analyzed for correlation relationships. With regard to VCs on 50 ppm HM-
472 amended plates and correspondent metal concentration, only a significant correlation between

473 Hg concentration and VCs on Hg-amended plates was detected ($p < 0.05$). By considering
474 VCs on 50 ppm HM-amended plates obtained in water and sediment samples, some strong
475 positive correlation was detected, such as stations 13W-13S, 18W-18S and 19W-19S ($p <$
476 0.05).
477 The relationships among physical, chemical and biological parameters for each sampling
478 station were analyzed by PCA that accounts for the variability within the data. This
479 multivariate analysis generated new variables, called principal components (PC; linear
480 components of the original variables). As it is shown in Figure 3, the first PC, representing
481 28.2% of the total variability, was mainly a mixture of chemical parameters, in terms of
482 pollutant concentration (PCB008, PCB028, PCB087, Cu, Hg, Tl). The second component,
483 which represented an additional 25.3%, was dominated by TCs and VCs on PCA, VCs on
484 HM-amended plates, and both HM and PCB concentrations.
485 Computed clusters based on Euclidean distances were superimposed to the PCA plots,
486 identifying that stations felt into three distinct clusters: *SEDI*, including the stations 1S, 8S,
487 5S, 15S, 16S, 17S, 18S and 19S; *SEDII* and *SEDIII*, composed only by the stations 9S and
488 13S, respectively. Station location seemed to have no influence on clustering. An additional
489 ANOSIM was computed with above definite station location as factors, confirming that no
490 significant differences occurred on the base of geographical position.
491 The ANOSIM Pairwise Test computed by setting clusters as factors showed a significant
492 difference between the three clusters ($p<0.02$). The SIMPER analysis underlined that the
493 higher average dissimilarity occurred between stations 9S and 13S, with higher cumulative
494 values for Cu, Cr, Co and PCB118 concentrations (cumulative values 84.2, 86.6, 88.9 and
495 91.2%, respectively), followed by *SEDI* and *SEDII* clusters, mainly due to VCs and PCB044
496 concentration (cumulative values 90.71 and 89.85%, respectively). Moreover, the distance
497 occurring among stations of the main cluster were due to VCs obtained on HM-amended

498 plates, in particular on Cu 500 ppm, Cd 50 ppm and Hg 50 ppm (cumulative values of 84.54,
499 88.37 and 92.22%, respectively).
500
501 3.3.2. Isolation of HM-tolerant bacteria
502 Bacterial colonies were isolated from the HM-amended plates used for VCs. A total of 111
503 HM-tolerant strains were isolated (96 and 15 from water and sediment samples, respectively)
504 from seven stations (i.e. 1, 9, 15, 16, 17, 18 and 19) (Table 5), with the majority of them that
505 were obtained from station 16W (73 isolates). HM-tolerant isolates were achieved from both
506 sediment and water only at stations 18 and 19. No isolate derived from Cd-amended plates.
507 Conversely, the majority of strains were isolated from Cu-amended plates (52 and 29 isolates
508 from 500 and 1000 Cu-amended plates, respectively), followed by Zn-amended plates (500
509 ppm; 31 isolates).
510
511 3.3.3. Multi-tolerant isolates
512 Isolates were screened for growth in the presence of metals different from that amending the
513 isolation medium. A total of 33 isolates (24 and 9 from water and sediment, respectively)
514 were selected due to their ability to grow in presence of more than one HM among those
515 tested at the concentration chosen as a threshold (i.e. 1000 ppm for Cu and Zn, and 100 ppm
516 for Hg and Cd; Table 6), and phylogenetically identified. They mainly belonged to the genus
517 *Pseudomonas* (25 isolates distributed in three OTUs), followed by *Pseudoalteromonas* (seven
518 isolates in a single OTU) and *Stenotrophomonas* (one isolate from 1S). Cu and Zn were
519 tolerated up to 1000 ppm, while Hg and Cd up to 100 ppm. Among isolates, ten *Pseudomonas*
520 isolates tolerated all tested metals, while additional five *Pseudomonas* isolates and
521 *Stenotrophomonas* sp. SMS1Z39 tolerated three metals. All remaining isolates, including

522 *Pseudomonas* (10 isolates) and *Pseudoalteromonas* (seven isolates) affiliates, tolerated only
523 two HMs.
524
525 *3.4. Enrichment of PCB-oxidizing bacterial isolates*
526 3.4.1. Viable counts on biphenyl-amended agar plates
527 VCs on BP-amended agar plates after enrichment were generally difficult to be determined as
528 bacterial growth occurred as a patina. Exception were stations 1S, 5S, 18S and 19S where
529 viable counts were 3.4±0.3, 47.2±17.5, 91.0±8.2, 17.2±2.2 x 10^2 CFU/g of wet sediment,
530 respectively.
531
532 3.4.2. Bacterial isolation and screening for PCB-oxidation
533 Bacterial colonies were isolated from the BP-amended plates used for viable counts. A total
534 of 362 strains were isolated and screened for growth in the presence of Aroclor 1242 at 4 and
535 15°C (Table 5). A total of 29 isolates were selected and phylogenetically identified (Table 7).
536 As for HM-tolerant isolates, PCB oxidizers mainly belonged to the genus *Pseudomonas* (18
537 isolates distributed in five OTUs), while the genera *Halomonas* (three isolates), *Pusillimonas*
538 (two isolates), *Achromobacter*, *Algoriphagus*, *Aeromicrobium*, *Arthrobacter* and *Rhodococus*
539 (one isolate each) were less represented.
540
541 3.4.3. Screening for the *bph*A gene
542 The *bph*A gene was harboured by 11 isolates, as follows: *Pusillimonas* sp. E17, *Halomonas*
543 spp. E24A and E24B, *Pseudomonas* spp. (seven isolates), and *Arthrobacter* sp. D21 (Table
544 7).
545
546 **4. Discussion**

547 Polar regions represent areas of great ecological valence, since they are particularly sensitive
548 to anthropogenic activity and the consequent pollution events, with deleterious effects on
549 abiotic and biotic resources. By virtue of their sensitivity, these areas are subjected to several
550 monitoring activities, such as the Arctic Monitoring Assessment Program (AMAP, 2011),
551 which report on the environmental impact of several stressors on Arctic organisms. The
552 observation and monitoring of freshwater sites acquire focal importance since they could be
553 considered as indicators of the damages occurred in the environment, and of the direct or
554 indirect effects on the biota and their development over time.
555 The Pasvik river area has been frequently monitored for the occurrence and concentration of
556 different pollutants in sediment and water (Dauvalter and Rognerud, 2001; Stebel et al., 2007;
557 Kashulin et al., 2009; Dauvalter et al., 2015; Dauvalter and Kashulin, 2018). Available
558 information indicates that the river is contaminated by a wide range of toxic and
559 bioaccumulative substances, including heavy metals (HMs) and polychlorinated biphenyls
560 (PCBs). Results on some HMs in water indicated that there had been no decrease within 6
561 years (Stebel et al., 2007). Dauvalter et al. (2015) reported lower HM concentrations in the
562 surface layer of the sediments from the area compared to deeper layers (2–4 cm). In addition
563 to HMs, persistent organic pollutants (POPs), such as PCBs, were detected in the river
564 sediment and water (Dauvalter et al., 2015). Among these, Christensen et al. (2007) reported
565 higher levels of POPs in Lake Kuetsjarvi compared to other lakes in the Pasvik area. In the
566 present study attention was paid to HM and PCB contamination in sediment samples.
567 Sediments are optimal matrices to be used for establishing contamination level in a certain
568 area, not only from a quantitative and qualitative point of view, but also as historical trace of
569 contamination. Obtained results confirm a high degree of contamination in the Pasvik river,
570 with remarkable amounts of HMs and PCBs that were generally determined in the middle and
571 inner areas of the river. Dauvalter and Rognerud (2001) investigated the contamination level

572 in the Pasvik river system, and reported high concentrations of some metals (i.e. Ni, Cu, Co,
573 Zn, Cd, and Hg) in the lower Pasvik River reaches, while a moderate contamination degree
574 was detected in the upper and middle ones. Here we observed higher levels of Mn, Zn, Ni and
575 Cu, but they were lower than those previously determined in the watershed of the Pasvik river
576 (Dauvalter and Rognerud, 2001). Stations 1S, 5S, 8S and 9S resulted the most polluted, but
577 this aspect seems to be not related to the geographical position, as supported also by the
578 statistical analysis, which didn't reveal significant differences among stations and areas.
579 PCB concentrations detected by Stebel et al. (2007) ranged from 10.5 to 36.4 ng/g, and
580 resulted higher than in sediments of other lakes in the same geographical area. In our case, the
581 highest concentration of PCBs were detected in sediments of stations 9S and 16S, ranging
582 from 0.01 to 126.64 ng/g, with a peak at station 9S, located in the middle sampling area.
583 Station 13S, with a total PCB congener concentration of 18.65 ng/g, was the station
584 characterized by the presence of most PCB congeners, sometimes totally absent in the other
585 sites.
586 The nMDS analysis computed on chemical data supports what was previously suggested, as
587 stations did not group in dependence of their position in the investigated area, suggesting that
588 concentration of individual metals and PCB congeners might be more likely related to local
589 inputs. For example, middle and (part of) inner stations in the Kirkenes area are heavily
590 influenced by intense vessel traffic, whereas high metal concentrations in the inner stations
591 probably derive from mining activities. Conversely, the lower amounts of metals often
592 determined at outer stations might be due to a greater dispersion acted by water circulation.
593 The occurrence of PCBs have been investigated in different Arctic matrices and ecosystems
594 (Evenset et al., 2004, 2007; Wang et al., 2009). In this work, stations 1S (outer) , 9S (middle),
595 16S and 13S (both inner) resulted the most polluted, with very high values that were generally
596 recorded for PCB008 (2,4'-dichlorobiphenyl) at almost all stations.

Microbial communities, as ubiquitarious and specialized components of the environmental biota, often suffer from impacting events that could strongly affect their functional activities. Pollutants act by altering the normal ecological equilibrium of these communities and, if they are chronically present or occur in large amounts, can induce microorganisms to develop adaptative responses. The development of resistance/tolerance towards xenobiotics thus makes bacteria useful bioindicators of polluted environments, and therefore they could represent a crucial tool in indirect monitoring approaches. Even if metal and PCB contamination of the Pasvik River area has been considered in the past, the bacterial response to the occurrence of these contaminants is here reported for the first time.

The observed order of tolerance (i.e. Cu>Zn>Cd>Hg) towards HM salts was congruent with that reported by Neethu et al. (2015) for Arctic bacteria isolated from water and sediment. Viable counts on HM-amended media were generally lower in water samples than in sediment samples, supporting the general assumption about the accumulation of pollutants, and the subsequent development of bacterial tolerance, in such matrix.

Bacterial viable counts on HM-amended plates generally reflected the HM contamination level detected in analyzed samples. This was particularly evident for station 19S, which was statistically detected as a source of significant higher bacterial abundances on 50 ppm HM-amended plates, and presented also the higher total amount of HMs (about 10865 ppb). The same was true for station 1S, which followed station 19S for total HM amount (about 10543 ppb) and bacterial abundance on 50 ppm HM-amended plates. Exceptions were represented by the inner stations 8S and 13S. Even if they were characterized by high concentrations of Cu (22.87 ppb at station 8S) and/or Zn (82.4 and 89.6 ppb at stations 8S and 13S, respectively) in sediment, a very low abundance of tolerant viable bacteria was observed. It is plausible to assume that such stations were subjected to a too recent contamination, so that tolerance had not been probably developed at sampling time.

622 HM-tolerant bacterial isolates were obtained from both water and sediment samples. In some
623 cases, the statistical analysis highlighted a positive correlation between VCs on 50 ppm HM-
624 amended plates of water and sediment samples, as well as at stations 13W-13S, 18W-18S and
625 19W-19S ($p < 0.05$). These stations were located in the middle and outer areas, and had a
626 greater degree of openness, so that they were probably more subjected to hydrodynamic
627 phenomena with dispersive effects on metals, leaving them suspended even in the water
628 column and not only deposited in sediments.

629 After subculturing, a higher number of HM-resistant isolates were achieved from water than
630 sediment samples (96 and 15 isolates, respectively), and tested for multitolerance. However,
631 the ability to tolerate more than one metal was more frequent in bacteria from sediment (9 out
632 of 15) than water (24 out of 96). Further, all multitolerant isolates derived from Cu or Zn-
633 amended agar plates and, among them, 23 were from water collected from the most internal
634 station 16W. This finding may suggest that bacteria adapted themselves to environmental
635 stressors by using different strategies, and that the tolerance properties may develop when
636 pollutant occurred in the bacterial habitat. This is in line with assumptions by Lo Giudice et
637 al. (2013) and Laganà et al. (2018), according to which pollutants like antibiotics and heavy
638 metals in polar environments primarily affect the bacterial communities.

639 Multitolerant strains mainly belonged to the genus *Pseudomonas*, followed by few members
640 in the genera *Pseudoalteromonas* and *Stenotrophomonas*. Several researches on HM resistant
641 bacteria have been conducted in the last several decades, and different sources of isolation
642 have been employed for the isolation of bacteria which exhibit such ability, therefore resulting
643 important in the cycles of these chemical elements (Castro-Silva et al., 2003; Otth et al.,
644 2005). However, literature on HM bacterial tolerance in the cold environment is very scant
645 (Neethu et al., 2015). González-Aravena et al. (2016) found metal tolerance ability in
646 *Psychrobacter* and *Pseudomonas* affiliated strains, isolated from an Antarctic sea urchin. The

647 authors highlighted the probable ship between presence of plasmids and resistance and co-
648 resistance to metals.
649 The potential of the genus *Pseudomonas* in HM tolerance have already been highlighted by
650 various authors, despite the strains originated from different matrices. For instance, Raja and
651 Selvam (2006) reported an excellent metal absorbing ability for a *Pseudomonas* strain isolated
652 from wastewater samples, and showed for the same strain multiple metal tolerance in
653 association with antibiotic resistance. To the best of our knowledge, data on cold-adapted HM
654 resistant *Pseudomonas* is very limited. Neethu et al. (2015) reported a higher toxicity of Hg, a
655 wide range of tolerance towards Cd and a good tolerance for Zn and Cu of a HM-resistant
656 *Pseudomonas* strains from the Arctic Kongsfjorden.
657 Finally, a *Stenotrophomonas* strain was reported as really promising in HM tolerance by
658 Tomova et al. (2014), who isolated resistant bacterial strains from soils in the East Antarctica,
659 and found that they were strongly resistant towards Pb, Ni and Cr. In addition, they detected
660 minimum inhibitory concentration up to 8.0 mM for Cu and multitolerance to six metals for a
661 strain closely related to *Stenotrophomonas rhizophila* BD17−E04. According to the authors,
662 the presence of multiple stressors leads bacteria to develop tolerance to each of them to
663 facilitate survival.
664 A correlation between HM tolerance patterns and the phylogenetic affiliation of isolates
665 seems to be suggested by our results, with *Pseudomonas* members that generally tolerated all
666 tested metals, and all *Pseudoalteromonas* isolates that tolerated only Zn (up to 1000 ppm) and
667 Hg (up to 100 ppm). However, *Pseudomonas* isolates, even if often grouping in the same
668 OTU, responded differently to the presence of heavy metals in the culture medium,
669 suggesting that tolerance was more likely strain- rather than species-specific and that single
670 species could probably tolerate different HMs due to different cell wall composition or
671 resistance strategies (Shruti et al., 2012). These latter certainly need to be improved in order

672 to better understand their possible involvement in environmental recovery strategies (Filali et
673 al., 2000; Malik, 2004). Another explanation could be that distinctive tolerance patterns might
674 be related to a possible resistance/tolerance acquisition or loss, for example *via* the transfer of
675 plasmid encoding resistance.
676 With respect to biphenyl-utilizing bacteria, higher bacterial densities were generally observed
677 on BP-amended plates inoculated with samples from inner and middle stations, where higher
678 PCB concentrations were determined. Interestingly, the higher VCs on BP-amended plates
679 were detected in samples with higher total PCB amounts, i.e. stations 9S and 16S. This could
680 indicate, as for metals, that the bacterial communities might be adapted to the occurrence of
681 such xenobiotics. This finding is also reinforced by the higher VCs that were determined in all
682 analyzed sediments, where such pollutants accumulate, than in water.
683 A total of 29 strains out of 362 bacterial isolates (8% of total isolates) were able to growth in
684 presence of Aroclor 1242 as the sole carbon and energy source. Such percentage is lower than
685 those reported by Papale et al. (2017), who reported a total of 21.5% for BP-degrading
686 bacteria from BP-enrichment cultures performed with sediments collected in the
687 Kongsfjorden, Arctic Norway. The percentage is also lower than the 21.4% of BP-degrading
688 bacteria observed for samples of Antarctic shallow sediments (Lo Giudice et al., 2013).
689 Nevertheless, results, suggest that the benthic bacterial communities in the Pasvik River
690 possess biodegradation capability. Cold-adapted isolates within the genera *Pseudomonas*,
691 *Arthrobacter* and *Rhodococcus* have been previously reported as PCB degraders (Mohn et al.,
692 1997; Master and Mohn, 1998; De Domenico et al., 2004; Papale et al., 2017), and they were
693 suggested as autochthonous PCB-degraders of Antarctic water column and sediment
694 compartments (Lo Giudice et al., 2013; Lo Giudice and Fani, 2015). Differently, *Halomonas*,
695 *Pusillimonas*, *Achromobacter*, *Algoriphagus*, *Aeromicrobium* members have been scarcely
696 described as PCB-degraders in cold regions. For instance, Papale et al. (2017) firstly reported

697 on an *Algoriphagus* strain as PCB-degrader from the Svalbard Islands. The presence of the
698 *bph*A gene confirmed the potential ability of 11 isolates to aerobically degrade PCBs. The
699 discrepancy between the presence/absence of such catabolic gene in isolates belonging to the
700 same OTU is not surprising as it could be related to methodological biases or explained by the
701 existence of several sequences of genes involved in catabolic functions (Furukawa et al.,
702 2004; Papale et al., 2017).

703

704 **5. Conclusions**

705 In conclusion, our results represent a contribute to the knowledge of the ecological and
706 environmental conditions of Arctic areas, which represent an important site for research and
707 human activities. The region here investigated showed a high rate of multiple pollution, in
708 which some sampling sites resulted most polluted than others. This was principally due to
709 local inputs rather than station position and pollutant accumulation along the River. A direct
710 influence of pollutants on the bacterial community distribution was observed. The ability
711 showed by psychrotolerant bacteria in tolerating multiple stressors, as such as HMs and
712 PCBs, underline their important role as self-depuration agents and, in particular, their
713 biotechnological potential at low temperatures. Beyond the interest in detecting resistant
714 bacteria for bioremediation applications, also the high sensitivity of bacteria to pollutants
715 could represent a good criterion for exploiting them as useful bioindicators. Further, as
716 suggested by Tomova et al. (2014), tolerant bacteria could be used to develop biosensors of
717 toxic metals in such sensible and strategic areas from an environmental point of view.

718

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727

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867 **Figure legends**

868 **Figure 1.** Map of the sampling area. The grey line represents the border limit between
869 Norway, Russia and Finland. Sampling sites are distinguished in Inner (▲), Middle (●) and
870 Outer (■) stations.

871 **Figure 2.** nMDS computed on heavy metal **(a)** and PCB (**b**) concentration determined in
872 sediment samples. ▲, Inner stations; ●, Middle stations; ■, Outer stations.

873 **Figure 3.** Principal Component Analysis (PCA) computed on HM concentrations and
874 bacterial viable counts in sediment samples. ▲, Inner stations; ●, Middle stations; ■, Outer
875 stations.