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Microbial response to human pollutants in polar lakes

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Sommario

Summary

Lakes are widely recognized as ecosystems that are highly sensitive to environmental changes, particularly small lakes and ponds that are abundant in the Arctic and Antarctica. These polar lakes, which cover large areas, especially in coastal regions, represent unique ecosystems rich in microbial life. Microbial communities play a critical role in nutrient cycling for both autotrophic and heterotrophic organisms. Even small variations in the dynamics and duration of snow and ice cover, or the deposition of xenobiotics, can significantly affect lake ecology. Bacteria rapidly respond to environmental changes, which can be studied by analyzing the expression of catabolic genes involved in the degradation or tolerance of organic and inorganic pollutants. Key results showed that bacterial communities, at the phylum level, did not vary significantly between the poles. Four main phyla predominated in lakes, both in water and sediment: Actinobacteriota, Bacteroidota, Alphaproteobacteria, and Gammaproteobacteria. However, Desulfobacterota were found in sediments at both poles, while Acidobacteriota were more prevalent in Arctic sediments. Arctic and Antarctic lake bacterial communities strongly differed at family and genus level. Among the metal-tolerant strains, those from the Arctic primarily belonged to Gammaproteobacteria, while Antarctic strains were mostly Actinobacteriota. Heavy metal sequestration tests revealed moderate abilities, although strains showed greater tolerance to iron, followed by mercury and copper. Regarding PCB degradation, nearly equal numbers of strains showed PCB oxidation ability, with the *bph*A gene identified in most strains. Most of these strains were identified as belonging to the genus *Pseudomonas*. Degradation tests indicated high efficiency across both poles, with the tetrachlorinated class of PCBs being the most degraded. In conclusion, the results represent a significant contribution to the biotechnological potential of polar bacteria for environmental remediation, highlighting the value of bacterial genera from polar lakes as potential alternatives to traditional processes.

1. Introduction

The polar regions (Arctic and Antarctic) cover about 14% of the Earth's landmass and constitute the largest part of the cryosphere, where water exists in a solid state. They (particularly Antarctica) play a crucial role in the Earth's climate system, as they act as a heat sink, absorbing and redistributing heat from the equator to the poles, and as a reflector, reflecting a significant amount of sunlight back into space, helping to cool the planet (Anisimov et al., 2007).

The Arctic spans approximately 7.1 million km^2 (4.8% of the Earth's surface), including the Arctic Ocean and its adjacent seas (the Nordic Sea and the Bering Sea), which comprise about two-thirds of the region. It also includes continental lands, consisting of the northern fringes of the continents of the Northern Hemisphere (such as Siberia in northern Asia, Scandinavia in Europe, Alaska, and northern Canada in North America) and islands, such as Novaya Zemlya (Russia), Svalbard (Norway), Iceland, and Greenland (Denmark), which is the world's largest island. A substantial discontinuity in the land exists only between Greenland and Norway. Other discontinuities between Asia and America (the Bering Strait) and between the islands of the Canadian Arctic Archipelago are of marginal significance. Much of the Arctic is low lying, except for the Greenland ice sheet, the ice-covered mountains of Ellesmere and Axel Heiberg islands, and the mountains in the northern part of the Beringia region (Przybylak et al., 2003). In contrast to Antarctica, the Arctic region is populated by 10 million people. With 415 settlements with a population of over one thousand people (Heleniak, 2020; Emelyanova, 2022). Furthermore, the Arctic is protected by laws regulated by the Arctic Council, composed of eight states that have sovereignty in this region: Canada, Denmark, Finland, Iceland, Norway, Russia, Sweden, and the United States.The Council's work has led to three legally-binding agreements: Agreement on Arctic Marine Oil Pollution Preparedness and Response, Agreement on Cooperation on Aeronautical and Maritime Search and Rescue in the Arctic, and an Agreement on Enhancing International Arctic Scientific Cooperation (Bloom, 1999; Canada, 2017) .

Antarctica, a vast continental landmass, is a unique environment that challenges life as we know it. It overlays the geographic South Pole and spans an area of approximately 14 million km^2, around 10% of the Earth's surface. From a biogeographic perspective, Antarctica is divided into two regions: continental Antarctica and maritime Antarctica (Convey, 2017; Singh et al., 2015). The former, located in the innermost part of the continent, is characterized by high latitude and altitudes and is covered by a layer of ice that reaches an altitude of more than 4,250 m above sea level, creating the most severe living conditions on the continent. The clearly defined maritime region encompasses the Antarctic Peninsula and the associated islands of the Scotia Arc and extends from the South Sandwich Islands through South Orkney and the South Shetland Islands, down the western side of the Antarctic Peninsula to approximately 72°S (Camacho, 2006). It experiences longer and warmer summers than the mainland, and is influenced by the Southern Ocean (Bölter et al., 2002).

Furthermore, the Antarctic is protected by laws regulated by the Antarctic Treaty, agreed by 29 States. The Antarctic Treaty System (ATS) governs the rights and obligations of states in respect of Antarctica and a comprehensive range of human activities in the region, from military activity and weapons testing, to scientific research, resource exploration and exploitation, tourism, fishing, waste disposal and wildlife conservation, among numerous other matters (Barrett, 2020; Molenaar, 2021).

1.1 Arctic and Antarctic lakes: extreme environments for life

Lakes and ponds are prominent features of the Arctic landscape and are also common in many parts of Antarctica. They represent transitory habitats in polar regions that can undergo rapid changes, from their initial formation to the eventual filling of the basin by abiotic and biotic sediments (Vincent et al., 2008) and even catastrophic draining when ice damming valleys are lost during glacial recession. Four characteristics of the physical environment distinguish polar aquatic ecosystems from those at lower latitudes: persistent cold-water temperatures, intense freeze-thaw cycles, prolonged ice cover, and the extreme seasonality of solar radiation and temperature regimes.

The polar regions are home to a remarkable diversity of lake types, ranging from freshwater to hypersaline, highly acidic to alkaline, and perennially ice-covered waters to concentrated brines that never freeze. These lakes exhibit different thermal regimes in summer, from fully mixed to thermally stratified, and various chemical characteristics, from oxygen supersaturation to anoxia, sometimes within the same lake over time or depth. Many polar lakes are ultra-oligotrophic or extremely unproductive but lakes highly enriched by animal or human activities can also be found (Vincent et al., 2008). Some lake types are exclusive to polar regions, such as solar-heated perennially ice-capped lakes and the epishelf lakes, highly stratified systems in which a layer of freshwater, derived from ice and snow melt, is dammed behind an ice shelf, defined as thick (>10 m), landfast (attached to the coastline) ice floating on the sea (Veillette et al., 2008).

Most polar lakes are characterized by prolonged, sometimes perennial, ice cover that is typically 3–6 m thick and may contain sand/rock flour and organic matter of aeolian origin (Miller & Whyte, 2011). Liquid water is present in the snow and ice profile during the summer melt period, especially when air temperatures are above zero, and large volumes of meltwater can be produced, forming channels that can transport nutrients and microorganisms into newly established proglacial lakes (Mindl et al., 2007). Thus, glacial meltwater has the potential to directly influence the composition of the microbial community in these lakes (Mindl et al., 2007). However, for many high-latitude lakes, liquid water persists throughout the year under thick snow and ice cover, and even some shallow ponds can retain a thin layer of water over their benthic communities in winter (Adrian et al., 2016). In other cases, polar aquatic habitats can stay relatively warm during winter. For example, shallow thaw lakes and ponds can warm to 10°C or more. The surface waters of northern lakes with high concentrations of dissolved organic matter that absorb light can experience daytime heating and temperatures > 15°C (Vincent et al., 2008). Such extreme conditions limit the distribution and the abundance of organisms that produce aquatic ecosystems dominated by microscopic species. However, given the proximity to the north-temperate zone, Arctic waters are characterized by a much more developed food web, with more diverse animal, plant, and microbial composition than Antarctica (Vincent et al., 2008).

High-latitude lakes hold significant global importance, serving as the habitat of unique species and communities, and sentinel of environmental change. Variations in snow and ice cover markedly affect all ecological variables (Quayle et al., 2002; Vincent et al., 2008). These aquatic ecosystems are particularly vulnerable to multiple stressors associated with local and global human impact, such as contaminant influxes, increased exposures to ultraviolet radiation, and climate change. Even small changes in their physical, chemical, or biological characteristics can lead to major shifts in their limnological properties and ecosystem structure and functioning (Flocco et al., 2019; Rosa et al., 2019; Ogaki et al., 2020; Quayle et al., 2002).

Polar lakes at high latitudes are already experiencing significant impacts from climate change, including the loss of perennial ice cover, leading to longer periods of open water conditions, increased water temperatures, stronger water column stratification, and changes in the balance of inflow and outflow. In some cases, these changes can lead to complete drainage or drying of lakes and swamps (Quayle et al., 2002; Vincent et al., 2008). The combination of these features makes polar lakes unique and interesting environments for studying the taxonomy and ecology of microbial communities living under extreme conditions. Understanding these ecosystems is crucial for predicting and managing the impacts of environmental change on these vulnerable systems.

1.1.1 Arctic lakes

A major feature of Arctic landscapes is their large number of lakes and ponds, which in some regions can cover up to 90% of the total surface area (Pienitz et al., 2008). Having the greatest water coverage of any terrestrial surface, the Arctic has been referred to as “the world’s largest wetland” (Fraser & Keddy, 2005). These numerous lakes and ponds are a striking component of Arctic regions, contributing significantly to Arctic biodiversity: they offer a diverse range of habitats for aquatic organisms, from microorganisms to animals and plants, and provide food and freshwater to migratory nesting birds, resident animals, and humans (Hoverman & Johnson, 2012).

The distribution of lakes and ponds in the Arctic is a complex interplay of various factors, including climate, geomorphology, substrate permeability, glacial history, and permafrost. These water bodies can be found on bedrock, surrounded by tundra, in areas of permafrost degradation, in glacier sediment, or on the glacial surface. The most common types of lakes are clear water lakes with no glacial inflow, meltwater lakes located near glaciers with a large amount of glacier runoff water, clear water lakes on the glacier surface (i.e., supraglacial lakes), and tundra and permafrost lakes (thermokarst) (Jeppesen et al., 2021).

Thermokarst lakes and ponds, typically shallow and formed by thawing ice-rich permafrost, are the most prevalent aquatic ecosystem type in the Arctic. They often form a complex of water bodies that are hot spots of biological activity in the tundra, hosting a wealth of microbes, benthic communities, aquatic plants, plankton and birds (Vincent et al., 2008). There are also significant differences in low and high Arctic lakes due to the effects of permafrost melting and degradation in lowland areas and mountains. Some lakes may also transition from glacier-affected turbid lakes to isolated clear-water lakes or vanish when they are no longer dammed by ice or cut off from glacier runoff water. Arctic lakes are typically oligotrophic, or even ultraoligotrophic (Burpee et al., 2016), and except were eutrophication is caused by ancient whaling residues (Douglas et al., 2020), sewage and wastewater (Rigler, 1972; Bennion et al., 2012; Kashulin et al., 2017), and more recently by increased geese and seabird populations (Hessen et al., 2017), eutrophic lakes are rarely mentioned in the Arctic scientific literature.

1.1.2 Antarctic lakes

The Antarctic continent and sub-Antarctic islands boast some of the most unique and captivating lake districts on Earth (Benninghoff, 1987). These lakes, predominantly located in coastal zones such as McMurdo Dry Valleys, Vestfold Hills, Larsemann Hills, Bunger Hills, Schirmacher Oasis and Syowa Oasis, and Schirmacher in continental Antarctica, as well as along the Antarctic Peninsula and

associated islands and archipelagos, offer a diverse range of formations and characteristics (Vincent et al., 2008; Phartiyal et al., 2011; Sokratova, 2011; Gonçalves et al., 2016; Ogaki et al., 2020).

Most Antarctic lakes are formed when the ice recedes, revealing depressions in the terrain, either from glacial erosion, the deposition of terminal moraines, or folds and depressions in the underlying geological topography (Hodgson et al., 2013). These lakes, with water temperatures typically remaining below 10°C, serve as a striking example of the region's extreme conditions. In winter, a thick ice cover between 1.5 and 6 m acts as a thermal barrier, preventing the freezing of the lake bottom (Hodgson et al., 2013). The perennial ice cover not only minimizes wind-generated currents and restricts light penetration but also decreases dissolved gas concentrations, causing heterogeneous sedimentation on the lake bottom (Wharton et al., 1989). The physicochemical features of these lakes range from some of the purest continental waters in the world to hypersaline lakes with salt concentrations eight times that of seawater, which maintain them in a liquid state, even during winter (Hodgson et al., 2013).

The nutritional status of Antarctic lakes is typically oligotrophic, with eutrophy generally restricted to those that are directly influenced by visiting marine mammals, birds or even humans (Shaw et al., 2023). These freshwater bodies, often in direct contact with rocks, soil, and mud, are home to a diverse range of vegetation, including Deschampsia antarctica, Colobanthus quitensis, mosses, macroalgae, and lichens (Ogaki et al., 2019). Despite their small size, the Microbial biota of these lakes is incredibly diverse and abundant, compensating for their lack of macroscopic life (Vincent et al., 2008; Hodgson et al., 2013).

The Antarctic Peninsula and Scotia Arc (maritime Antarctic) present lakes and ponds formed in the rocky substratum as well as coastal lagoons influenced by marine water intrusions (Camacho, 2006). They are typically shallow, transparent, receive high amounts of light and ultraviolet radiation, are cold, and generally have low nutrient availability, characteristics that represent limiting factors for the resident microbiota (Ellis-Evans, 1996). However, large and deep lakes also exist, e.g., on Fildes Peninsula in King George Island.

In these areas, the climate is less extreme than in the continent's interior, so in summer, although temperatures are generally below 0 °C, ice melting and liquid precipitation are typical. These conditions lead to a more active hydrological cycle with sediment and nutrient circulation throughout the lacustrine basin (Quayle et al., 2002; Camacho, 2006).

In the maritime Antarctic, most lakes originated from glacial retreat, and a few have been formed by tectonic and volcanic activity (Priddle & Heywood, 2008). Some lakes, such as the Crater Lake at Deception Island, are formed in volcano areas or volcanic depressions, which contain deposits of pyroclastic material (Ogaki et al., 2019). To date, volcanoes are still active in the South Shetland Islands, and in Kroner Lake on Deception Island, fumarole activity supplies heat to the lake, which makes it a unique geothermally heated lake in Antarctica (Priddle & Heywood, 2008).

Most of the lakes in continental Antarctica show a typical concentration of ions and solutes, which is acquired when glacial meltwaters meet soils and sources surrounding them, making them saline or hypersaline (Green & Canfield, 1984).

Antarctic oases of the continental area are ice-free regions located in relief depressions or old marine lagoons where the presence of unfrozen water (in the form of a system of seasonal streams and non-freezing lakes) makes them unique landscapes (Sokratova, 2011; Shevnina & Kourzeneva, 2017). The lakes and ponds found in these areas are shallow and relatively warm, but some large, deep, and cold-water lakes are generally covered with ice, 3–5 m thick, throughout the year (Matsumoto, 1993; McKay et al., 1994; Doran et al., 1996). They occur in tectonic faults, depressions of valleys, or in proximity to glaciers (Sokratova, 2011; Shevnina & Kourzeneva, 2017). Most lakes typically have high-density stratification and hypersaline bottom water, probably formed during glacial low stands. The salt composition of lake waters of the Antarctic oases is formed because of the transport of marine aerosols by precipitation, salt freezing, and ion inflow from the upper layers of the soils of lake basins (Sokratova, 2011). Nutrients are concentrated in the anoxic bottom waters because of the lack of circulation, producing an oligotrophic status of the lakes and ponds (Bishop et al., 1996). However, these extreme habitats host active biosystems: microbial mat communities that can flourish in the lake

bottom sediments, owing to the absence of a significant foraging fauna (Bird et al., 1991; Bishop et al., 1996). Antarctic oases host a great variety of lake systems: more than 150 hypersaline and low-salinity lakes, formed by the retreat of the ice from ice sheets (Bird et al., 1991), are found in Vestfold Hills on Princess Elizabeth Land at the margin of the East Antarctic ice sheet; more than 100 freshwater lakes, ranging from small ponds to deep lakes (Stüwe et al., 1989; Shevnina & Kourzeneva, 2017) are located in Larsemann Hills, that consist of several ice-free peninsulas and islands along the coast; few perennially ice-covered lakes at Bunger Hills (Doran et al., 1996), one of the largest ice-free areas on the Antarctic continent, located near the coast of East Antarctica at about 100° longitude, covering an area of about 300 km^2 and consisting of low rocky hills and glacially deepened valleys (Sheraton et al., 1993). Most of the lakes are in the center of the oasis and thus become ice-free in the summer months, while the lakes at the edge of the oasis, in contact with glacier ice, mostly retain their ice covers throughout the year (Doran et al., 1996).

Lakes of continental Antarctica are generally epiglacial, i.e. they are found on the surface of the ice sheets, glaciers, and ice shelves; however, there are active and partially interconnected subglacial hydrological systems connecting more than 379 lakes that exist beneath the Antarctic ice sheet (Wright & Siegert, 2012). These lakes lie up to 4200 m under the Antarctic ice sheet and range in size from 1 to 241 km long (Hodgson et al., 2004). They are subject to high pressure (approximately 350 atmospheres), low temperatures (about −3 °C), and permanent darkness, characteristics that make these lakes some of the most extreme environments on Earth. Lake Vostok is the best-known subglacial lake in Antarctica, continually buried under glacial ice for 15 million years (Rogers et al., 2013). It lies between 3750 m (at the south of the lake) and 4150 m (at the north) beneath the central-east Antarctic ice sheet (Siegert et al., 2001) and is the largest and deepest lake in East Antarctica (240 km long, 50 km wide).

1.2 Microbial life strategies in polar environments

The extreme physical conditions in Arctic and Antarctic environments not only challenge the activity and survival of living organisms, including prokaryotes (Bacteria and Archaea) but also provide a unique opportunity to study the remarkable adaptations of these organisms. Many prokaryotes have adapted to survival at subzero temperatures and endure the harsh and changing climate. However, at freezing temperatures, reduced enzyme activity may affect metabolic functions in prokaryotes, and water may form damaging ice crystals in the cells. Additionally, the presence of extracellular ice may limit the diffusion and transport of vital solutes and nutrients. Physical stress may also occur from exposure to intense solar radiation, low air humidity, and reduced water availability at some sites (Holmberg & Jørgensen, 2023).

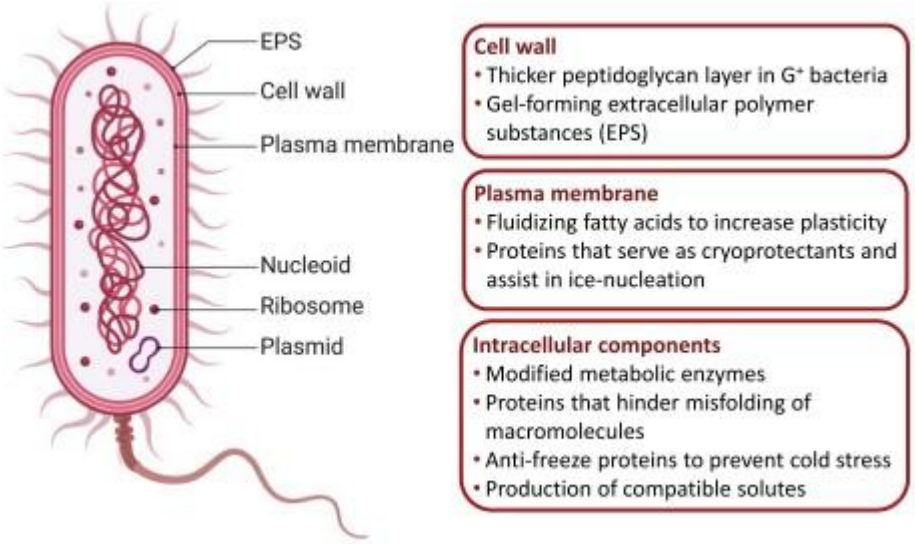


Fig. 1: Psychrophilic adaptation strategies in microorganisms (Holmeberg and Jørgensen, 2023).

Prokaryotes have not just survived but thrived in low and subzero temperatures through a series of ingenious cellular modifications. These adaptations, which may include changes in the cell wall, the cell (or plasma) membrane, and intracellular components, as summarized in Fig. 1, are a testament to their resourcefulness. The cellular adaptations can comprise the following changes:

- Gram-positive bacteria, such as *Bacillus subtilis*, have been found to thicken their cell walls by increasing the content of peptidoglycan. This adaptation improves protection from disruption due to ice formation, freeze-thaw cycles, and/or increased osmotic pressure. In addition, extracellular polymeric substances can be produced that create a hydrated gel to inhibit ice formation and desiccation, and the formation of a protective biofilm may also be stimulated (Holmberg & Jørgensen, 2023).
- The cell or plasma membrane can be modified to (i) increase its plasticity by the addition of fluidizing fatty acids; (ii) include a higher content of cryoprotectants (proteins, glycolipids, and pigments); and (iii) contain ice-nucleating proteins that form extracellular ice crystals and prevent the development of larger crystals (Sharma et al., 2022).
- Intracellular components can be modified to ensure correct cellular functions and may include: (i) Optimization of enzymes to improve substrate uptake and function at low temperatures; (ii) Chaperone proteins that counteract misfolding of proteins and stabilize secondary structures of RNA and DNA to preserve their primary structure and function; (ii) Cold-shock proteins that prevent freeze damage but may have additional functions to improve tolerance to various other stresses, e.g., osmotic and starvation stresses; (iv) Anti-freeze proteins that bind to tiny ice crystals and prevent ice formation as well as inhibit further crystal formation; and (v) Production of compatible solutes (various organic molecules) that protect the cell from dehydration and shrinkage by restoring the osmotic balance (Baek et al., 2012).

1.2.1 Prokaryotes inhabiting polar lakes

In polar freshwater lakes and ponds, the prokaryotic diversity is strongly influenced by environmental variations that control ecological 'species-sorting' processes (Crump et al., 2003; Leibold et al., 2004; Judd et al., 2006; Fierer et al., 2007; Jones & McMahon, 2009). Generally, the bacterial communities present in polar lakes are dominated by Proteobacterota, Bacteroidota, and Actinomycetota both in

the water column and at the rock–water interface (Michaud et al., 2012; Huang et al., 2013; Papale et al., 2017; Picazo et al., 2019). Less abundant but commonly found phyla include Chloroflexota, Acidobacterota and Firmicutes. The proximity of a lake to the shoreline is a key indicator of its nutrient availability and bacterial abundance, with inland lakes typically being shallower and receiving negligible influxes of organic matter from fauna. For example, Chloroflexota found in lake water have been strongly associated with penguin colonies, which contribute to the community composition of shoreline lakes through the influx of organic matter (Picazo et al., 2019). Sea sprays are also a source of organic inoculation for near-shore lakes, typically resulting in higher salt content and the presence of marine microbial species. A large share of the detected lake Proteobacterota comprises some ultra-oligotrophs and diazotrophs, specifically species belonging to the families Bradyrhizobiaceae and Burkholderiaceae (Picazo et al., 2019). These oligotrophs are much less represented in lakes with higher nutrient availability, generally shoreline lakes. Betaproteobacteria are especially prevalent in the upper layers of most oligotrophic Antarctic lakes and are represented by genera such as *Polaromonas*, *Polynucleobacter*, *Rhodoferax*, and *Limnohabitans* (Li & Morgan-Kiss, 2019). The genera *Flavobacterium* and *Pseudarcicella* dominated Bacteroidota especially in shallow lakes (Michaud et al., 2012; Picazo et al., 2019). Since *Flavobacterium* has been implicated as part of microbial mat communities, it stands to reason that sediment-associated bacteria significantly impact lake water community composition in shallower lakes (Li & Morgan-Kiss, 2019; Picazo et al., 2019). For the Arctic the most ubiquitous phyla are Actinobacteriota, Betaproteobacteria, Bacteroidota, and Alphaproteobacteria (Crump et al., 2012). In the study by Comte et al., (2018), the genus *Polaromonas* was found to be the most representative. According to the authors, the presence of this genus indicates a strong influence of organisms from snow. Other genera were reported in a study by Le Moigne et al. (2020) and included *Polynucleobacter*, *Variovorax*, *Rhodoferax*, *Planktoluna*, *Limnohabitans*, *Rhodoblastus*, uncultured Burkholderiaceae, *Rhodoluna*, Candidatus *Planktophila* (aclA), and *Methylorosula*. In some extreme lake conditions such as in perennially ice-covered lakes, where the effect of these seasonal habitat shifts is pushed to

the limit, the microbial community structure is represented by a general shift from photoautotrophy to chemolithotrophy (Vick-Majors et al., 2014). More specifically, Alphaproteobacteria annotated OTUs had the highest autumn proliferation, along with some archaeal OTUs, which could be linked to chemolithotrophy and active methanogenesis. Still, the phyla Actinobacteriota and Bacteroidota remained dominant for most of the year. Dispersal also influences the biogeography of microbial species (Martiny et al., 2006; Telford et al., 2006), but the mechanisms of dispersal and the scales at which they are relevant are poorly understood. It is becoming clear that patterns of microbial diversity are controlled by both dispersal and environmental conditions, including biological interactions, but what remains unclear is the relative importance of these two factors and how they interact on different temporal and spatial scales (Crump et al., 2012).

1.3 Contamination of polar regions

The widespread perception is that the Arctic, being situated far from all major cities and man-made activities, and Antarctica, being a continent physically isolated by the Southern Ocean, remain clean and pristine. Unfortunately, the 'pristine' concept is no longer accurate as these regions are not free of pollution. The presence of contaminants that originate from local and global sources is well documented. They include inorganic as well as organic compounds (Szopinska et al., 2017). Inorganic contaminants consist predominantly of heavy metals (HMs). The organic contaminants include persistent organic pollutants (POPs) and pesticides.

Several heavy metals including iron (Fe), cobalt (Co), copper (Cu), manganese (Mn), zinc (Zn), and molybdenum (Mo) crucial for metabolic activity at low concentrations and are considered essential element micronutrients (Peralta-Videa et al., 2009) and may play an essential role in metabolic activity, e.g., metalloenzyme (Tahir et al., 2017). However, when the concentrations of heavy metals exceed a certain threshold, they produce adverse effects (Ali et al., 2013). In general, high concentrations of all heavy metals present toxic effects. However, certain heavy metals including

arsenic (As), cadmium (Cd), lead (Pb), mercury (Hg), and chromium (Cr) are non-threshold toxins and are described as the most problematic heavy metals. Although a portion of the heavy metals in the polar environment is introduced from natural sources, the majority originates from a variety anthropogenic of activity, both at local and remote scales (Planchon et al., 2002; Mishra et al., 2004; Hur et al., 2007). Research stations, especially in Antarctica, mining (as example in Svalbard, High Arctic), tourism, electricity generation, transportation, and garbage management (Bargagli, 2008) and associated activities such as sewage outfalls, abandoned dump sites, accidental oil spills and exhaust emissions are the primary local sources of heavy metal contaminants (Aronson et al., 2011). These elements are among those released by the combustion of coal, oil, and gasoline and high metal concentrations have been detected in abandoned dump sites or areas affected by scattered rubbish or emissions from incinerators, generators and vehicles (Kennicutt et al., 1995, 1995; Claridge et al., 1995; Webster et al., 2003). For example, in Antarctica, before relevant environmental legislation was developed and applied in the late 1980s and 1990s, anthropogenic waste was often simply disposed into landfill sites or the adjacent sea (Cunningham et al., 2005). Although earlier waste dumping sites in Antarctica have been closed, many have not been cleared, and pollutants including heavy metals are still detectable to the present day (Chu et al., 2019). Heavy metal contaminants released from human activity from around the globe, such as metallurgy, industry, fossil fuel combustion, waste incineration and transportation are entering global circulation and reach the Arctic region and the Antarctic continent via long-range oceanic and atmospheric transport (Truzzi et al., 2017; Chu et al., 2019). These contaminants can be transported over long distances owing to their relatively inert nature, low water solubility, and low deposition velocity. While long-range oceanic transport may be significant for soluble heavy metals (Cd, Pb) (Macdonald et al., 2005; Maccali et al., 2013), long-range atmospheric transport is crucial for heavy metals released in gaseous form. For example, Mercury (Hg) is emitted into the atmosphere from several natural as well as anthropogenic sources. Gaseous Hg is relatively stable in the atmosphere and can be transported through air currents far from the place of its origin. However, the reaction between sea salt, sunlight and atmospheric

mercury can transform gaseous Hg into more reactive mercury. Once deposited in terrestrial and aquatic ecosystems, this reactive Hg is partly re-emitted into the air, thus assuming the characteristics of global pollutants such as persistent volatile chemicals (Sing & Tiwari, 2008). Animals represent another natural source of heavy metal contamination; animal-impacted sites are often areas with naturally elevated levels of heavy metals. For instance, it has been recently reported that orthogenic soils from penguin rookeries in the Antarctic Peninsula and South Shetland Islands are characterized by high contents of Cd, Cu and As. Penguins are important in the biotransportation of heavy metals, resulting in high concentrations of Cd, Cu and As in Antarctic soils (Santamans et al., 2017). Through the guano of penguins, heavy metals are transported from marine to terrestrial ecosystems in Antarctica (Espejo et al., 2014). In addition, penguin guano in sediments from the Antarctic Peninsula was found to be enriched with As, at concentrations double those of the background sediments (Xie & Sun, 2008). Animal carcasses are another important natural source of heavy metal contamination. For instance, seal carcasses are a significant source of Hg and methylmercury contamination in Antarctic soils (Zvěřina et al., 2017).

Polychlorinated biphenyls (PCBs) are part of a larger group of persistent organic pollutants (POPs) which are released into the environment through a variety of human activities and have a range of harmful effects on the health of ecosystems, wildlife and people. Theoretically, the family of PCBs consists of 209 related compounds, called congeners, consisting of a biphenyl carbon ring substituted with one to ten chlorine atoms (chemical formula: $C_{12}H_{10}$-nCln). The half-life of PCBs in the environment also depends on the number of chlorines in the biphenyl structure. It can range from a few days to approximately ten years for mono- to pentachlorobiphenyls, reaching more than twenty years for higher substituted congeners (Borja et al., 2005; Ceccarini & Giannarelli, 2007). Large amounts of PCBs have legally or illegally entered the environment for decades through accidental spills, inappropriate disposal techniques or a variety of thermal and chemical processes with no consideration for their impact on biota. Since the 1970s, when their strong toxicity and recalcitrant nature were recognized, PCBs have been regulated or banned in Western nations because of the

serious health and ecological problems potentially deriving from their widespread utilization. This has led to worldwide contamination, even in remote areas, such as Arctic and Antarctic regions. The question of the fate and transport of pollutants in remote areas have received increased attention in the last years and the polar regions, which in the past were considered untouched areas, in terms of anthropogenic pollution, have become a topic of great concern as the Arctic environment is far from pristine and Svalbard are perhaps one of the areas most affected by anthropogenic pollution transported from industrialized areas (Sander et al., 2006). The marine transportation contributes modestly to the processes of pollutants long-distance spread, while the global mechanism underlying these processes of diffusion is atmospheric transport on a hemispherical scale (Jianmin et al., 2016).

Organic pollutants tend to volatilize with extreme simplicity to high temperatures and can travel, thanks to heliotropic movements, significant distances until they meet a little colder climatic zone in which, condensing, fall to the ground. The Arctic environment is, therefore, an important receptor for different groups of persistent toxic substances from countries both North and South of the world, because of its special climate and so it is a real trap (Ma et al., 2016). The substances that come up to the Arctic are accumulated at both flora and fauna level, since there remain in the ecosystem due to their persistence and their high lipophilic nature. The degradability of pollutants decreases more slowly with cold, on the contrary of bioaccumulation that increases. At the North Pole high concentrations of pollutants were found in seals (Troisi et al., 2020), polar bears (Bytingsvik et al., 2012) and the Inuit population (Adamou et al., 2020) living in those lands.

1.3.1 Presence of heavy metals in Polar lakes

Different studies have reported the presence of metals in Arctic lakes. In a study of four lakes in Alaska, found the presence of high concentration of Cu, Ni, and Zn in all the sediment lakes studied and attesting a high presence of Cd, Hg and Pb in almost one lake (Allen-Gil et al., 1997). In another study conducted on seven lakes near the city Murmansk (Arctic Russia), the water concentrations in

the different lakes were compared to the background levels, highlighting for example that Ni concentration showed 2.6 – 11 times above the background, Cu concentration was 0.7 – 1.6 times higher to the background and the concentration of Fe ranged from 36 to 2684 μg L^{-1} (the maximum values was 27 higher than the permissible concentration for fisheries in this area) (Postevaya et al., 2021). Even in the same region, the authors affirmed that the analysis of the distribution of concentrations of Ni (up to 127 mg/kg), Cu (up to 370 mg/kg), Pb (up to 90 mg/kg) revealed the influence of long-range atmospheric pollution on lakes of the Natural Park of Rybachy and Sredny Peninsulas (Slukovskii et al., 2024). Finally, in four lakes near the Ny-Alesund (Svalbard) the concentration detected in sediment lakes were as follows: Cd (0.58–2.63 μg/g), Pb (below detection limit–42.51 μg/g), Cu (20.73–219.39 μg/g), Cr (7.47–18.64 μg/g), and Zn (33.83–57.16 μg/g) (Rajaram et al., 2023).

In Antarctica, a study of Grovnes & Broknes peninsula, Larsemann Hill, East Antarctica in 60 lake sediments detected the following metals: Al from 0.01 to 0.79 μg L^{-1}, Fe from 0.04 to 3.27 μg L^{-1}, Mn from 0.18 to 12.83 μg L^{-1}, Zn from 0.59 to 2.44 μg L^{-1}, Cu from 1.01 to 5.37 μg L^{-1}, Ni from 0.21 to 0.60 μg L^{-1}, Cr from 0.09 to 0.82 μg L^{-1} (Bhardwaj et al., 2023). Another study in 16 lacustrine lakes situated in the central Dronning Maud Land (cDML) region of East Antarctica detected the avarege of the followed metals: Al (77,504.09 ppm), Cd (1.36 ppm), Co (29.52 ppm), Cr (102.75 ppm), Cu (65.19 ppm), Fe (57,632.87 ppm), Mn (679.05 ppm), Ni (49.13 ppm), Pb (10.11 ppm), and Zn (253.78 ppm) and affirmed the main are originated from natural weathering of source rocks (Magesh et al., 2021). Finally, in a study in a Lake 55 from Schirmacher Oasis situated along the Queen Maud Land in East Antarctica. The average concentration of the elements varies in the following order: Al>Ca>Fe>Na>Mg>Ba>Mn>Zn>Sr>Cr>Co>Ni> Pb>Li>As, with the following concentration As between 3.01 and 10.11 mg/kg, Ba 521.35 to 1116.72 mg/kg, Pb 15.33 to 24.95 mg/kg, Zn 88.42 to 366.25 mg/kg, affirmed also in this case that metal concentration derived from natural weathering of source rocks (Joju et al., 2024).

1.3.2 Metal resistance mechanisms in bacteria

In bacteria, heavy metal toxicity also depends on their concentration and availability, even if some metals like silver (Ag) and Hg are poisonous at very low concentrations (Lemire et al., 2013). To avoid cellular damage, bacteria have evolved regulatory mechanisms of resistance to metals. In 1960, the first bacteria was detected that was metal-resistant, when Hg-resistant (HgR) bacteria (*Staphylococcus aureus*) were isolated from a wound (Pal et al., 2017). Some 42 studies showed evidence that heavy metals can induce co-selection of antimicrobial resistance in bacteria (Baker-Austin et al., 2006; Seiler & Berendonk, 2012). In 2016, Lloyd and her team found that bacteria resistant to three or more antibiotics were more common in HgR isolates than in Hg sensitive (HgS) isolates (Lloyd et al., 2016). Fig. 2 shows the three main metal resistance mechanisms known in bacteria (Lemire et al., 2013; Seiler & Berendonk, 2012):

1. Extracellular sequestration of metals, which minimizes the concentration of free metal ions in the cell;
2. Reduction of metal uptake and increased elimination of toxic metals by efflux systems;
3. Inactivation of metals through the reduction of intracellular ions by enzymes like the Hg reductase (MerA) which reduces Hg ions (Hg^{2+}) to the less toxic form, the elemental Hg (Hg^{0});
4. Repair mediated by cellular chaperones, enzymes or antioxidants of molecules that are vulnerable to oxidation by metals.

It was also observed that metal resistance shares a common mode of action that also confers antibiotic resistance (Sanseverino et al., 2018). The association between antibiotic and metal resistance is a pressing issue in the field of microbiology. Co-selection mechanisms, where genes encoding resistance to both metals and antibiotics may reside in the same genetic element (co-resistance), or when there is a coregulation of resistance genes expression (co-regulation), or again when a single mechanism is responsible for the induced resistances (cross-resistance), are areas of active research. Multidrug efflux pumps, which can extrude a variety of compounds, including antibiotics and heavy

metals, mainly through cross-resistance mechanisms, are a key focus. An example is the multidrug efflux pump in *Listeria monocytogenes*, which can export metals and antibiotics. Fang et al. (2016) described that in Escherichia coli strains isolated from diseased food-producing animals, genes encoding efflux systems to detoxify Cu, Ag and As co-existed with antimicrobial and heavy metal resistance determinants on the same plasmids, giving an example of co-resistance. Co-resistance of Hg-resistant bacteria (HgR) to antibiotics has been observed in a study where the Hg exposure in fish increased the expression of the Hg reductase gene (merA), providing resistance to Hg and showing a higher probability for these bacteria to be resistant to multiple antibiotics compared to the Hg-sensitive bacteria. As an example of co-regulation, a study performed in *Pseudomonas aeruginosa* showed that the expression of an efflux system conferring resistance to Zn, Cd and cobalt (Co) was regulated by mechanisms also responsible for the resistance to carbapenems (Perron et al., 2004). A study in China found a significant positive correlation between antibiotic resistance genes (ARG) and metals like Cu, Zn, and Hg in agricultural soil and manure, showing the potential role of heavy metals in the co-selection of antibiotic resistance. However, in the same study, the correlation between ARG and the corresponding antibiotic concentration was much weaker (Ji et al., 2012).

In a study from the Antarctic lakes near Maitri base camp, about the bacteria isolate (mainly Flavobacterium spp. and *Pseudomonas* spp) exhibited multiple metal resistance (Hg, Cd and Zn). In the same study, *Aeromonas* showed resistance to mercury and chromium (De Souza et al., 2007).

Instead, in Arctic strain isolate from Pasvik River belonged to the genus, *Pseudomonas, Pseudoalteromonas* and *Stenomonas* showed the multitolerance of Cu, Zn, Hg and Cd (Rappazzo et al., 2019). These findings underscore the urgent need for further research and understanding of the role of bacteria in the resistance to heavy metals and antibiotics (Knapp et al., 2017).

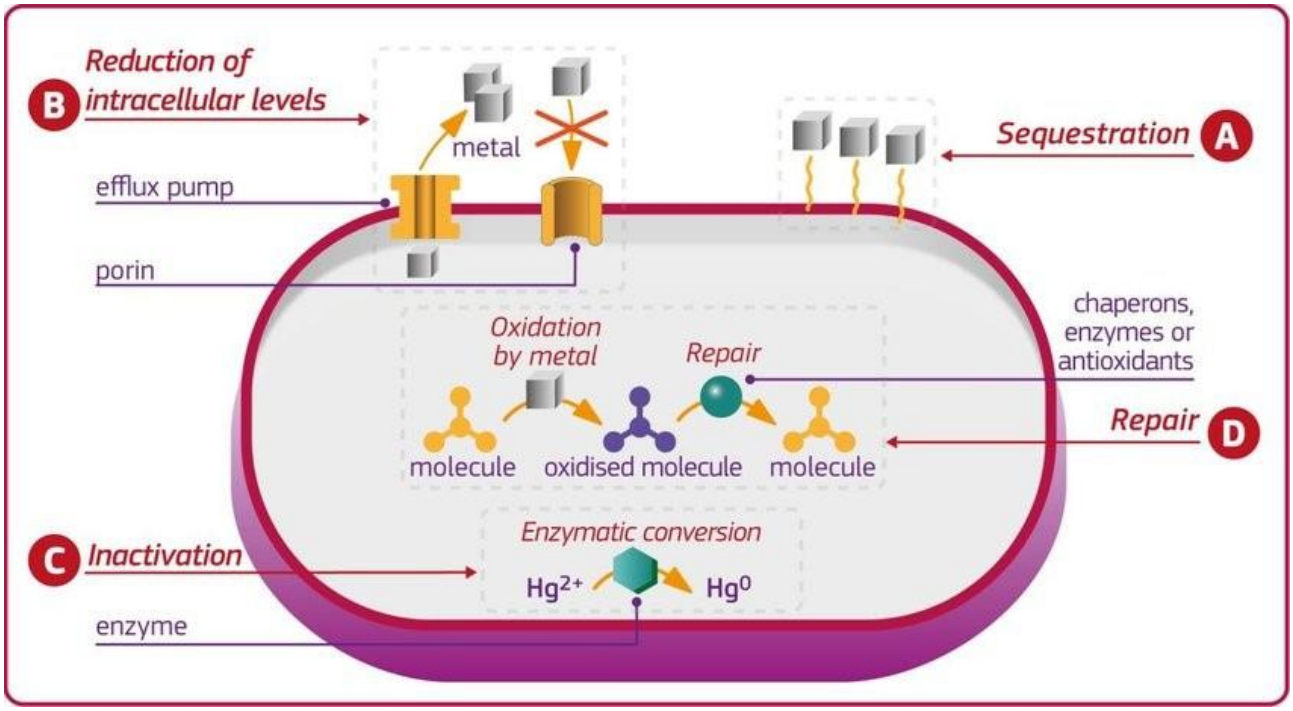


Fig. 2: Metal resistance mechanisms in bacteria. (Figure from Sanseverino et al., 2019).

1.3.3 Presence of policlorobiphenyls in polar lakes?

In the literature, several studies about PCBs in polar lakes were presented, highlighting the prevalent issue of PCB contamination in these delicate ecosystems. The first study in Antarctica was done in 1982 by Tanabe et al. (1983), who found 48–610 pg L^{-1} in ice and water from a lake near the Japanese Antarctic station. Subsequently, a study from 1988 to 1992 detected 120 pg g^{-1} in a sediment lake from Victoria Land (Fuoco et al., 1996). Another study in James Ross Island found that the level of PCBs ranged between 0.32–0.83 ng g^{-1} in sediment lakes with a prevalence of tri- and tetrachlorinated congeners (Klánová et al., 2008). Finally, in a study about the lakes in Victoria Land, the PCB detected in water was 46 – 143 pg L^{-1}, instead of in sediment, ranging between 10 – 634 pg g^{-1} (Vecchiato et al., 2015). In the Arctic, the concentration of PCB is more elevated than in the Antarctic region. In the lakes affected by birds in the Norwegian Arctic, the concentration of PCB in the water was 23 – 129 pg L^{-1} (Evenset et al., 2007). Instead, in the sediment of a remote lake in the Canadian Arctic, 2.56 ng g^{-1} was detected, with a prevalence of tri-tetra- and pentachlorinated (Stern et al.,

2005). In seven sediment lakes in West Greenland, the concentration detected was 61 – 2619 pg g^{-1} (Malmquist et al., 2003). In a study of surface water from the Fuglebekken basin near the Polish station at Honsund, Svalbard Island, the PCB concentration varied from 2.3 to 406 ng L^{-1}. (Polkowska et al., 2011). On Svalbard Islands, we examined six Ny-Ålesund lakes, which were detected with levels ranging from 0.18 to 13 ng g^{-1} (Jiao et al., 2009).

1.3.4 PCB degradation by bacteria

Microorganisms can degrade PCBs through the meta-cleavage pathway, producing Krebs cycle intermediates and chlorobenzoates (Pieper, 2005). In aerobic biodegradation, the primary step is the dioxygenation of PCB congeners, catalyzed by the enzyme biphenyl dioxygenase. This enzyme facilitates the incorporation of two hydroxyl groups into the aromatic ring of the PCB congener, making it more susceptible to enzymatic ring-cleavage reactions (Brühlmann & Chen, 1999). Biphenyl dioxygenase is a multicomponent enzyme composed of a terminal dioxygenase (with a large alpha and a small beta subunit), ferredoxin, and ferredoxin reductase, encoded by the *bph* operon (Erickson & Mondello, 1992). The *bphA* gene, which encodes the large alpha subunit of the enzyme, is involved in substrate recognition (Fig. 2). This *bphA* enzyme is substrate-specific and varies among organisms. The presence of the *bphA* gene has been reported in *Pseudomonas aeruginosa* JP-11, isolated from Paradip port, Odisha, India, with 98.86 ± 2.29% efficiency in biphenyl degradation (Brühlmann & Chen, 1999; Chakraborty and Das, 2016). It has also been observed in *Burkholderia cepacia* and *Pseudomonas pseudoalcaligenes*, showing sequence similarity in their *bph* operon. However, in *Burkholderia cepacia*, biphenyl dioxygenase preferentially deoxygenates ortho-substituted PCBs, whereas in *Pseudomonas pseudoalcaligenes* KF707, the enzyme primarily deoxygenates para-substituted PCBs (Erickson & Mondello, 1992). The *bphA* gene was found also in *Lysobacter, Salinibacterium, Planococcus, Pusillimonas* and *Arthrobacter* all isolate from the Antartic Lakes from Edmonson Point (Papale et al., 2022). Also, in freshwater environment, from

Pasvic River were found the strains belong to *Pusillimonas sp., Halomonas spp.*, *Pseudomonas spp.* and *Arthrobacter sp.* had the gene *bphA* (Rappazzo et al., 2019).

DNA shuffling experiments in *Burkholderia cepacia*, *Comamonas testosteroni*, and *Rhodococcus globerulus* produced enzyme variants with enhanced PCB degradation capabilities ((Furukawa & Fujihara, 2008). This method involved in vitro random recombination of *bphA* genes by fragmentation and PCR reassembly. It was observed that the hybrid BphA, II-9 (a hybrid of *B. cepacia* and *C. testosteroni*) was able to oxygenate 2,6-dichlorobiphenyl up to 58% after 18 hours, while the parental enzymes performed this reaction 10% less efficiently (Barriault et al., 2002). Using a rational design approach, biphenyl dioxygenase from *P. pseudoalcaligenes* KF707 was three-dimensionally modeled. This model was based on crystallographic analysis of the naphthalene dioxygenase enzyme from *Pseudomonas sp.* (Suenaga et al., 2002). A clear visualization of key positions near the enzyme's active site was obtained, which was used for site-directed mutagenesis. Consequently, mutants of *Pseudomonas* 1335F, T376N, and F377L efficiently degraded 2,5,2′,5′-tetrachlorobiphenyl, a compound that could not be mineralized by the wild-type biphenyl dioxygenase enzyme (Ang et al., 2005). Typically, the primary product of PCB degradation is chlorobenzoate, which requires specific catabolic plasmids from other microorganisms for its cleavage. *Pseudomonas aeruginosa* harboring the plasmid pE43 contains the oxygenolytic ortho-dechlorination *gene ohb*, while Arthrobacter globiformis with the plasmid pPC3 carries the hydrolytic para-dechlorination *gene fcb*. Transformation of *Comamonas testosteroni* with these recombinant plasmids enabled the utilization of ortho- and para-chlorobiphenyl as sole carbon sources (Hrywna et al., 1999). Similarly, 4,4′-dichlorobiphenyl (a double para-substituted congener) was easily mineralized by *P. pseudoalcaligenes KF707*, but 2,5,2′,5′-tetrachlorobiphenyl could not be degraded. *B. cepacia LB400* has broad substrate specificity, attacking 2,5,2′,5′-tetrachlorobiphenyl via 3,4-dioxygenation (Suenaga et al., 2001). Amino acid mutations in enzymes also influence substrate specificity. In the *bphA* enzyme, asparagine (Asn) at position 376 confers broad substrate specificity, whereas enzymes with narrow specificity contain threonine (Thr) at this site. The replacement of Thr-376 with Asn in

P. pseudoalcaligenes KF707 BphA introduced 3,4-dioxygenase activity for the degradation of 2,5,4′-trichlorobiphenyl and 2,5,2′,5′-tetrachlorobiphenyl (Kimura et al., 1997). In naphthalene dioxygenase, this amino acid corresponds to Thr-351 in the large subunit. However, replacing Thr-351 with Asn in this enzyme had a negligible effect on product formation (Parales et al., 2000). Earlier reports suggested that four amino acid substitutions in *P. pseudoalcaligenes* KF707 BphA increased its degradation ability for single aromatic compounds such as alkylbenzenes, benzene, and toluene (Suenaga et al., 1999). Therefore, site-directed mutagenesis-based structural modelling of enzymes can provide steric information leading to altered specificity and function. PCB degradation has been reported in bacterial species like *Acidovorax, Bacillus, Burkholderia, Comamonas, Corynebacterium, Cupriavidus, Pseudomonas, Rhodococcus*, and *Sphingomonas* (Furukawa and Fujihara, 2008; Seeger et al., 2009). *Burkholderia xenovorans* LB400 was capable of degrading a broad range of PCBs and has been considered a model bacterium for PCB degradation (Seeger et al., 2010). *Rhodococcus jostii* RHA1 was another potent PCB-degrading soil bacterium (McLeod et al., 2006). In the presence of biphenyls and chlorobiphenyls, PCB-degrading bacteria accumulate polyphosphates (polyP) during their exponential growth phase as an adaptive mechanism. *Pseudomonas* strain B4 and *B. fungorum* LB400 accumulated polyP to enhance survival in the presence of chlorobiphenyls (Chavez et al., 2004). Thus, it can be concluded that pollution stress drives the synthesis of certain stress-resistant adaptations in bacteria for survival in toxic-contaminated sites. Additionally, the design of engineered proteins with modified active sites can aid in the enhanced bioremediation of toxic organic pollutants.

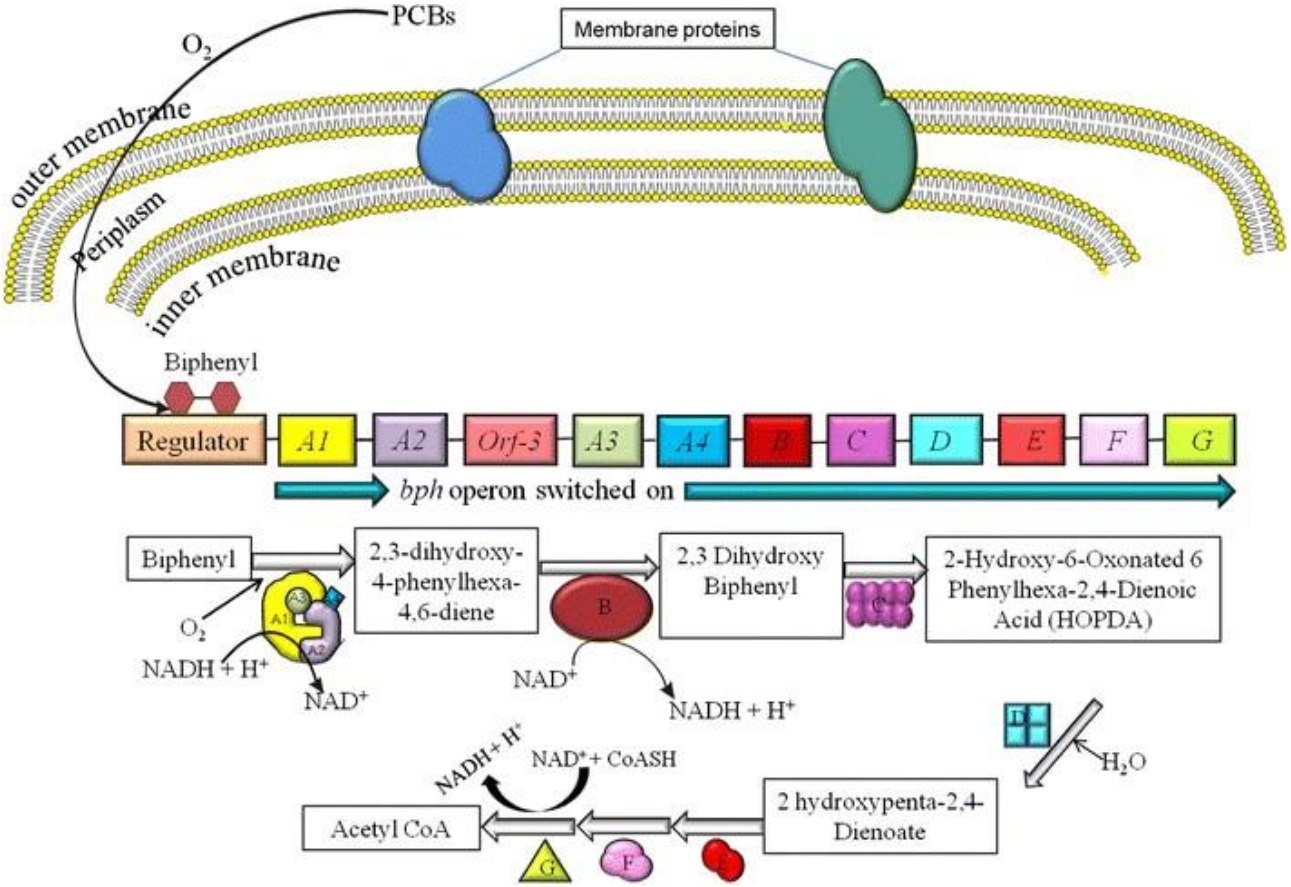


Fig 3: *bph* operon is the genetic way at the base of PCB degradation in bacteria. It's composed of different genes that code for the protein complex involved in the processes of oxidoreductase (genes A1, A2, A3, A4, B, and C), hydrolysis of a chemical bond (genes D, E, F), and phenols' degradation (gene G) (from Chakraborty and Das, 2016)**.**

2 Aim of the work

The purpose of this thesis was to study prokaryotic communities inhabiting Arctic and Antarctic lakes, both in water and sediment, examining their ecological diversity and isolating bacteria capable of degrading PCBs or tolerating heavy metals. To achieve this, three main objectives were envisaged:

1) Analysis and estimation of prokaryotic community biodiversity in polar lake water and sediment by molecular analyses.
2) Enrichment, isolation and characterization of PCB-degrading or heavy metal-tolerant bacterial strains, and estimation of their biodegradative efficiency at low temperatures.

3) Correlation of results from the prokaryotic communities with chemical contamination to gain an overview of the microbial community adaptation and specialization, due to the pollutant pressure on the environment.

Results and discussion will be reported in separate chapters according to the different research objectives, while a general conclusion on the entire data set will be presented at the end of the document.

3 Materials and methods

3.1 Sampling area and sample description

Two sampling campaigns were carried out in the framework of the MicroPolArS "Microbial response to human Pollutants in polAr lakeS" project funded by the Programma Nazionale di Ricerche in Antartide (PNRA 18_00194; PI Maria Papale). One was in Ny-Ålesund in the Arctic and the other one in Antarctica in the South Shetland Islands archipelago.

3.1.1 Ny- Ålesund

Ny-Ålesund (78°55' N, 11°56' E), Svalbard, Arctic Norway, is one of the world's northernmost settlements situated on the Brøgger peninsula, in the north-west area of Spitsbergen, the largest of the Svalbard Islands. Ny-Ålesund is warmer and more humid than elsewhere with a similar latitude in the Northern hemisphere, due to atmospheric circulation and the oceanic cycle in the North Atlantic and the Barents Sea (Yuan et al., 2010). The mean temperature is around -14 °C in the coldest month (February) and 5 °C in the warmest month (July) (Jiang et al., 2011). Ny-Ålesund is surrounded by a variety of High Arctic ecosystems typical of Svalbard, and most of the islands' animal and plant species can be found in this area, making the settlement an ideal starting point for researching the flora and fauna of the Arctic Circle. Ny-Ålesund was a former Norwegian coal mining town that was

closed in the 1960s due to a tragic accident (Hisdal, 1998). Now, there are small number of permanent residents (scientific expeditioners and logistical support personnel), and the population climbs to more than one hundred in summer. Because of its unique climate and geographical location, Ny-Ålesund is thought to be an ideal location for Arctic scientific exploration. Thus far, several research stations have been established in the area.

Water and sediment samples were collected between 5 and 18 August 2021 from five lakes surrounding the Ny-Ålesund research village: Solvannet (L1), Glacier (L2), Knudsenheia (L3), Storvatnet (L4) and Tvillingvatnet (L5) (Fig. 4).

Solvatnet is located close to the Ny-Ålesund Post Office and behind the Indian research station Himadri; Glacier is close to the Vestbreen glacier; Knudsenheia lies roughly 3 km away from Ny-Ålesund in the north-west direction; Storvatnet is located about 1 km west of Ny-Ålesund and south of the airstrip in Hamnerabben; Tvillingvatnet is located about 1.5 km south-west of Ny-Ålesund. All lakes' surfaces are frozen during the winter and melt in summer. Geographical coordinates and temperature, pH, dissolved oxygen, conductibility, and temperature of water physio-chemical data for each sampling point are shown in Table 1. For each lake, sediments were collected in three points almost equidistant for set up an enrichment. For the analysis of the bacterial community water and sediment were collected in one point.

Tab 1: Geographical location and physio-chemical data for each sampling point.

Area	Lake	Sample ID	Coordinates		Physio-chemical parameters			
					Water temperature (°C)	pH	O_2 %	Cond (uS/cm)
Ny-Ålesund (High Arctic Norway)	Solvannet	L1-1	N 78°55.552'	E 11°56.327'	6.2	8.17	98.0	395
		L1-2	N 78°55.555'	E 11°56.574'	6.7	8.16	102.2	422
		L1-3	N 78°55.508'	E 11°56.326'	6.7	8.25	102.1	379
	Glacier	L2-1	N 78°55.044'	E 11°47.442'	8.7	7.66	98.2	151
		L2-2	N 78°55.036'	E 11°47.671'	9.1	7.90	98.8	153
		L2-3	N 78.55.060'	E 11°47.508'	8.7	8.00	99.0	147
	Knudsenheia	L3-1	N 78°56.680'	E 11°51.579'	8.8	8.36	105.3	2620
		L3-2	N 78°56.650'	E 11°52.083'	8.8	8.39	106.7	2680
		L3-3	N 78°56.735'	E 11°51.235'	9.4	8.46	107.5	2680
	Storvatnet	L4-1	N 78°55.453'	E 11°52.728'	7.9	8.07	103.2	243
		L4-2	N 78°55.476'	E 11°52.263'	7.9	8.06	101.3	246
		L4-3	N 78°55.392'	E 11°52.824'	7.9	8.13	103.5	234
	Tvillingvatnet	L5-1	N 78°55.058'	E 11°51.922'	7.9	7.71	102.3	227
		L5-2	N 78°54.988'	E 11°52.164'	8.2	7.83	102.7	220
		L5-3	N 78°54.954	E 11°52.739'	8.3	7.91	102.4	220
Livingston Island (Antarctica)	Sofia	LS-1	S 62°40'12.19"	W 60°23'17.90"	0.3	5.2	69.4	26.5
		LS-2	S 62°40'13.61"	W 60°23'21.36"	0.9	5.6	87.8	26.1
		LS-3	S 62°40'11.84"	W 60°23'09.85"	-0.2	5.6	96.24	8.8
	Argentina	LA-1	S 62°40'22.39"	W 60°24'18.12"	1.4	5.7	68.1	64.6
		LA-2	S 62°40'23.76"	W 60°24'17.08"	0.6	5.64	66.2	67.5
		LA-3	S 62°40'11.84"	W 60°23'09.85"	-1.4	5.35	63.7	63.7
Deception Island (Antarctica)	Crater	LC-1	S 62°59'00.68"	W 60°40'20.41"	3.7	5.5	86.0	6.64
	Zapatilla	LZ-1	S 62°59'00.24"	W 60°40'29.07"	6.8	5.6	76.5	55.6
		LZ-2	S 62°59'00.99"	W 60°40'32.24"	6.8	5	92.8	106.7
	Extremadura	LE-1	S 62°55'12.2"	W 60°39'47.0"	4.1	7.2	94.8	448
		LE-2	S 62°55'05.8"	W 60°39'40.6"	3.9	6.3	84.4	510
	Telefon	LT-1	S 62°55'39.9"	W 60°41'21.3"	5.4	6	-	507
		LT-2	S 62°55'42.1"	W 60°41'29.0"	6.8	5.8	-	477
		LT-3	S 62°55'44.4"	W 60°41'21.4"	9.9	6.3	-	419
	Ballaneros	LB-1	S 62°58'51.1"	W 60°34'27.1"	4.1	4.7	67.9	480
		LB-2	S 62°58'49.6"	W 60°34'46.1"	4.7	3.3	95.6	509
		LB-3	S 62°58'44.92"	W 60°34'36.30"	9.32	2.8	87.9	280

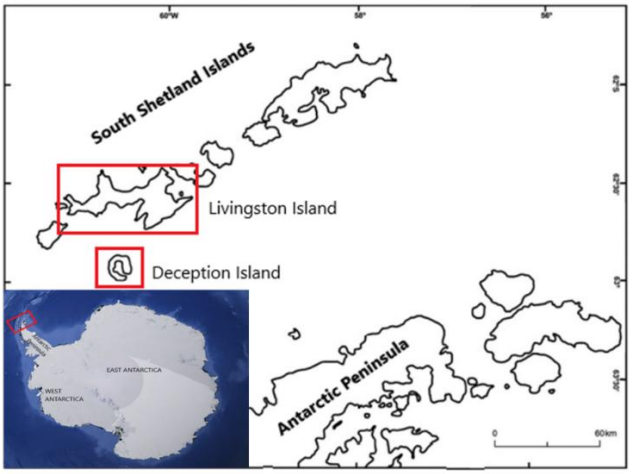
Fig 4: Maps showing Svalbard Islands and the sampling locations in the Ny-Ålesund area.

3.1.2 Deception Island and Livingston Island

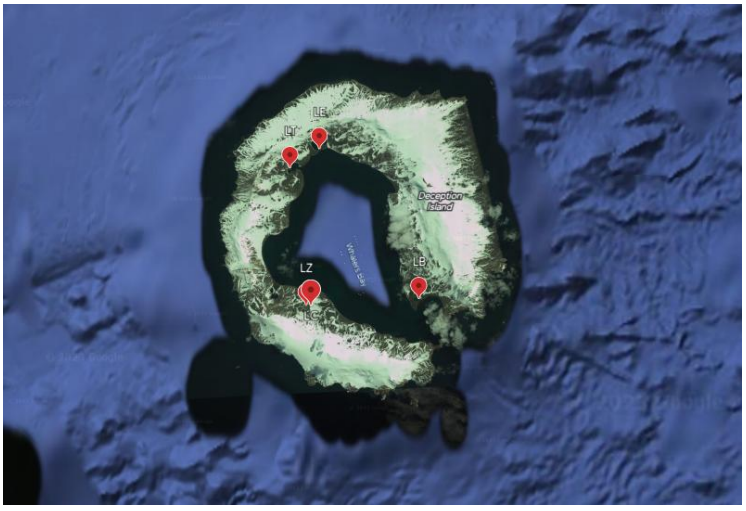
Deception Island (62°57'S, 60°38'W) is located in the South Shetland Islands archipelago, that lies in the Bransfield Strait, 100 km north of the Antarctic Peninsula. It is one of the few active volcanos in Antarctica, with recent eruptions in 1967, 1969 and 1970 (Baker et al., 1975). It is a horseshoe-shaped basaltic island, surrounding an internal flooded caldera, Foster Bay, of 7 km diameter, which was formed by an explosive eruption c. 10 000 years ago. The island is 15 km at its in maximum diameter, rising to an altitude of 539 m at Mount Pond, and 452 m at Mount Kirkwood, both of which are covered by permanent ice. About 57% of the island is covered by ice caps, glaciers and ice-covered moraines, pyroclasts and ashes from volcanic activity (Smith, 2005). Its climate is typical of the northern maritime Antarctic with a long cold summer with mean monthly air temperatures of 0–2.5 °C from November–March and 0 -10 °C from April–October. Precipitation throughout the year (c. 900 mm) is mainly as snow, but summer rainfall, fog and low cloud are frequent. The island experiences little sunshine and frequent strong winds (Smith, 2005). There are freshwater and geothermal springs on the island, as well as several lakes, freshwater pools, streams and a geothermal

lagoon (Downie et al., 2000). Livingston Island (62°34'35" S - 60°54'14" W), the second largest island of the South Shetland Islands (974 km^2), is located in the Southern Ocean at 110 km from the Antarctic Peninsula and 830 km from the tip of South America (Cape Horn). Most of the island is covered by glaciers and icecaps, while only 10% is ice-free during summer. The largest of these ice-free zones is Byers Peninsula, the most western tip of the island (Toro et al., 2007). The peninsula is bordered to the east by Rotch Ice Dome, which reaches a height of approximately 360 m. Apart from the northwestern part of the peninsula, which reaches 268 m above sea level, few areas reach altitudes higher than 100 m except for some residual hills (usually volcanic plugs) (Björck et al., 1991; Toro et al., 2007). Livingston Island has a maritime climate, less extreme than on the Antarctic Continent with temperatures between 1 and 3 °C in summer, with a maximum that can even reach 10 °C and a minimum that can reach -10 °C. In winter the minimum temperature can drop to -35 °C and the maximum generally does not exceed 0 °C (Toro et al., 2007). Rainfalls are more abundant than the rest of the Antarctic continent, with annual averages between 700 and 1000 mm wet equivalent of snow (Bañón, 2001). The human presence on the island is limited to the permanent scientific bases of Juan Carlos I (Spain) and St. Kliment Ohridski (Bulgaria) which were established in 1988 at South Bay and the small Chilean Shirreff Base. Water and sediment samples were collected between 25 January and 1 February 2022 from 7 lakes: Argentina (LA) and Sofia (LS) (both in Livingston Island) and Ballaneros (LB), Crater (LC), Extremadura (LE), Telefon (LT) and Zapatilla (LZ) (all in Deception Island) (Fig. 5).

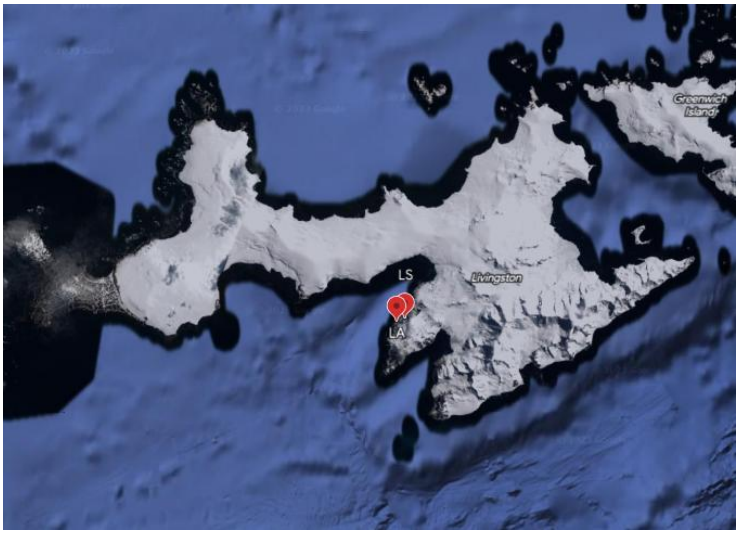
For each lake, were collected sediment in three poin almost equidistant for set up an enrichment, except for the Crater, Extremadura and Zapatilla Lakes in Antarctica from which one or two sampling points were considered due to difficulties in sampling, For the analisis of the bacterial community were collect water and sediment in one point (Tab. 1).



a)



b)



c)

Fig. 5: Maps showing a) Deception and Livingston Island and, in detail, the location of lakes sampled in b) Deception Island and c) Livingston Island.

3.1.3 Collection and preliminary treatment of samples

For the molecular study of the prokaryotic community, 1 to 3 L of the water samples was filtered on polycarbonate membranes (diameter 47 mm; 0.22 µm pore size), in triplicate; they were then immediately frozen at −20 °C for transport to Italy. Sediment samples were collected in the same points of each lake at a depth of 30–60 cm. The first 10 cm of superficial sediment were sampled using a pre-cleaned scoop and sterile plastic containers; they were then transported to the laboratories of the research stations for immediate storage at -20 °C.

Two different procedures were followed for set up the enrichments, i.e. the first for heavy metals (Rappazzo et al., 2019) and the second one for biphenyl (Papale et al., 2017). In the case of heavy metals, enrichment from each sediment samples (L5 excluded) was set up using 1 g of wet sediment to inoculate 75 mL of filter-sterilized lake water (collected from the same site at sampling time), containing the following heavy metal salts, individually: $FeSO_4$ (Fe), $CuSO_4$ (Cu) and $HgCl_2$ (Hg) (Sigma), at a final concentration of 1000 ppm for Fe and Cu, and 100 ppm of Hg.

For the biphenyl (BP) enrichment, BP was used as the sole carbon and energy source for microbial growth. Aliquots (1 mL) of a stock solution (75 mg mL^{-1}) of BP dissolved in chloroform were added to empty Erlenmeyer flasks, and the solvent was allowed to evaporate completely. Subsequently, 1 g of wet sediment was used to inoculate 75 mL of sterile water of the same lake in BP-containing flasks (final concentration 0.1%, wt/vol). All the enrichments were stored at 4 °C, until in Italy.

3.2 Bacterial community biodiversity

3.2.1 Total DNA extraction and NGS target sequencing

DNA was extracted from environmental samples using the DNeasy® PowerSoil® Pro Kit (Qiagen) according to the manufacturer's instructions. Briefly, each sample (membranes and 1 g of sediment) was added inside to appropriate tubes (PowerBead Pro) where both the detachment and cellular lysis,

due to mechanical action of the beads and buffer chemical reactions, took place. Using different solutions, it was possible to separate the microorganisms from filter membranes or sediment particles and to facilitate cell lysis. The genomic DNA was subsequently trapped in a silica membrane, present inside the MB Spin Column, which was then subjected to a series of ethanol-based washes. The DNA obtained was resuspended in 100 µL of Milli-Q water and stored frozen at -20 °C for subsequent analyses. DNA concentrations and purity were quantified by NanoDrop ND-1000 UV–Vis spectrophotometer (NanoDrop Technologies, USA). The bacterial 16S rDNA region V3-V4 was amplified using universal primers 27F (5' – AGAGTTTGATCACGGCTCAG – 3') and primer 1492R (5' – TACGGYTACCTTGTTACGAC – 3'). Sequencing was performed using the Illumina MiSeq platforms, following the standard protocols of the company EurofinsEurope Services (Germany).

3.2.2 Bioinformatics analysis

FastQC was used to check the quality of raw sequences (Brown et al., 2017). Sequences were preprocessed, quality filtered, trimmed, de-noised, merged, modeled, and analyzed by R package DADA2 (Weißbecker et al., 2020) to infer amplicon sequence variants (ASVs), i.e., biologically relevant variants rather than an arbitrarily clustered group of similar sequences. During the analysis, filters for reducing replicate, length, and chimera errors were also applied. Bacterial taxonomy annotation was performed using the SILVA SSU database 138 formatted for DADA2. Finally, a manual inspection was done, and sequences with 0.1% abundance were considered together in the minor groups of retrieved bacteria.

4.2.3 Statistical Analyses

To compare the bacterial community compositions across groups of samples, the Bray–Curtis similarity analysis was performed and similarity matrices were used to obtain dendrograms using R

base packages. Principal component analyses (PCAs) were performed using the factoextra R package, on data from selected physical and chemical properties of sediment and water, heavy metal concentration, POP concentration and the relative abundance of significant bacterial taxa. Environmental variables used in these analyses were as follows: oxygen (O_2%), temperature (°C), conducibility (Cond uS/cm) and pH. The Spearman' ρ correlation (performed in R software, R-base packages) was used to obtain connections between retrieved factors and environmental parameters, results were considered significant when the *P* value was less than 0.05. However, before this analysis, the data were log-transformed to linearize the relationships and avoid the influence of magnitude.

3.3 Isolation of heavy metal tolerant bacterial strains

After incubation, aliquots (100 µL) of each enrichment were spread plated, in duplicate, on Nutrient Agar (NA) together with the respective HMs used for the enrichments (Fe, Cu or Hg), separately, at the same concentrations (1000 ppm for Fe and Cu, and 100 ppm for Hg). NA plates without metals were used as negative controls. Plates were incubated at 4 °C for 30 days. For bacterial isolation, colonies were randomly selected from agar plates, picked, and subcultured on NA under the same conditions.

3.3.1 Test of growth with increasing concentrations of HMs

Each isolated strain was subsequently tested for growth at increasing concentrations (up to 5000 ppm for Fe and Cu, and up to 500 ppm for Hg) with the same HM added to the isolation medium. For this, a growth test in 96-well microtiter plates (Labsolute) was set up, and the optical density (OD) of the bacterial cultures was measured over time, using a modified version of the protocol described by Blasi et al. (2016). Starting cultures were prepared by growing each bacterial strain in a Microtiter plate. Each well was filled with 250 µL of nutrient broth (NB) medium, in duplicate. The plates were

then incubated at 4°C for 15 days. Growth was assessed daily by measuring OD using a microplate reader (Adsorbance 96 spectrophotometer, Byonoy, Germany) at λ = 605 nm (OD605). The recorded values were processed using the Absorbance 96 software. Wells containing NB without bacterial inoculation were used as blanks for the OD readings. After incubation, 25 µL of each strain culture were transferred, in duplicate, into 225 µL of 10% NB amended with HMs at the following concentrations: 2500 ppm and 5000 ppm for Fe and Cu, and 250 ppm and 500 ppm for Hg. The plates were then incubated at 4°C for 15 days. Growth was assessed daily as described above. Wells containing NB10% medium with HM but without bacterial strain inoculation were used as blanks for the OD readings.

3.3.2 Screening for HM multi-tolerance

Bacterial strains capable of growing in the presence of higher concentrations of HMs were then assayed for tolerance to different HMs then those used in original the enrichment cultures. For multi-tolerance testing, the selected strains were streaked onto NA amended with HMs and incubated for 30 days at 4°C. The concentrations used were: 1000 ppm, 2500 ppm, and 5000 ppm for Fe and Cu, and 100 ppm, 250 ppm and 500 ppm for Hg, respectively (Rappazzo et al., 2019).

3.3.3 Sequestration rate of HMs

To test the HM sequestration rate, selected bacterial strains were grown in broth medium (NB) spiked with 190 ppm of mercury or 2250 ppm of iron. Two negative controls were prepared: one containing only NB and the other with NB and the additional HM. After incubation for 30 days at 4°C, the samples were centrifuged at 8000 rpm for 20 minutes at 4°C to separate the liquid phase from the pellet. The samples prior analysis were diluted (1:1000) in a solution of one liter containing 0.1% of Triton X, 1% of nitric acid, and 0.1% of EDTA, before analysis by Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES; characteristics of the instrument in Tomasello et al., 2024) following the method described by Turetta et al. (2021).

3.4 Isolation of PCB-oxidising bacterial strains

Aliquots (100 µL) from each enrichment were plated on solidified bushnell haas (BH). BP was added as crystals (0.1 g) to the Petri dish lid after inoculation. Replicate plates were incubated at 4 °C for 30 days. BH agar plates without BP were used as a control. For bacterial isolation, colonies were randomly selected from the agar plates, picked, and subcultured on nutrient agar medium.

3.4.1 Bacterial growth in the presence of PCBs

Bacterial strains isolated from BP enrichments were screened for PCB degradation under aerobic conditions. Isolates were inoculated in BH amended the PCB mixture Aroclor 1242 (100 ppm in dichloromethane, CH_2Cl_2) as the sole carbon and energy source (final concentration 0.1 %, v/v) (De Domenico et al., 2004; Michaud et al., 2007; Lo Giudice et al., 2013; Papale et al., 2017). Aroclor 1242 is a mixture of PCB congeners (ranging from dichloro- to hexachlorobiphenyls) consisting of twelve carbon atoms in the biphenyl molecule and containing 42 % chlorine by weight. Before adding the bacterial inoculum, 10 µL of Aroclor solution was transferred to 100 mL flasks containing 9.5 mL of BH and left in a cabinet until all the CH_2Cl_2 completely evaporated.

The medium was inoculated with 0.5 mL of a freshly prepared bacterial suspension. Cultures were incubated at 4 °C for 3 weeks on a rotary shaker operated at 100 rpm. The ability to use PCBs as growth substrates was evaluated according to the degree of turbidity or the appearance of cellular flocs in the test tubes. Uninoculated medium was incubated in parallel as a negative control (Lo Giudice et al., 2013).

3.4.2 Screening for the presence of the *bphA* gene

Strains showing the ability to grow in the presence of Aroclor 1242, as sole carbon and energy source, were screened for the presence of the catabolic gene *bph*A involved in PCB degradation (Master & Mohn, 1998). The presence of genes was highlighted by a modified protocol of Lehtinen et al. (2013)

through PCR, using specific primers selected by previous analyses: 2BPHFWD1 (5' ADVCCSCGBGCCGCBTCHTCG 3') and 2BPHREV1 (5' ADVCCSCGBGCCGCBTCHTCG 3'). The reaction mixtures were assembled at 0 °C and contained 1 µL DNA, 0.5 µL of each of the two primers (10µM), 2.5 µL of reaction buffer 10x, 0.025 µL of BSA (2.5%), 0.2 µL of *Taq* polymerase Bioline (5U/µL), and were made to volume with sterile Milli-Q water to a final volume of 10 µL. Negative controls for DNA extraction and PCR setup (reaction mixture without a DNA template) were also used in every PCR run. The PCR program was as follows: 1) 95 °C for 5'; 2) 35 cycles at 95 °C for 45 ", 58 °C for 1' and 72 °C for 2'; 3) 72 °C for 10'. The results of the amplification reactions were analyzed by agarose gel electrophoresis (2%, w/v) in TAE buffer (0.04 M Tris-acetate, 0.02 M acetic acid, 0.001 M EDTA), containing 1 µL SYBR™-safe solution (Fermentas) each for 25 mL of gel.

3.4.3 Degradation efficiency assay

Isolates were grown in 50 mL of Aroclor 1242 supplemented (1%, v/v) BH and incubated at 4 °C for 1 month (Papale et al., 2017). Uninoculated controls were incubated in parallel to monitor abiotic losses of the substrates. After incubation, biodegradation activity was stopped by acidifying the cultures with HCl 10 M to achieve pH 2. Before PCB extraction, the cultures were centrifuged for 20 minutes at 8000 rpm at 4 °C to remove the excess of cellular material. Afterwards 5 µL of octachloronaphthalene (OCN) solution (5 mg/mL in CH_2Cl_2) was added to each aliquot to monitor substrate losses during the extraction procedure. Cultures were extracted three times with 10 mL of CH_2Cl_2 in a separatory funnel to isolate the organochlorine molecules from the enrichment cultures. Funnels were vigorously shaken for 15 min during each extraction. The three organic phases were pooled, and the solvent evaporated to dryness.

The composition of the PCBs and their concentration was determined by high resolution gas chromatography-mass spectrometry (GC-MS), using an 7890A-5975C (Agilent Technologies) equipped with a 60-m HP-5MS column (0.25 mm I.D., 0.25 µm; Agilent Technologies, Avondale,

USA). Helium was used as carrier gas (1.2 mL min^{-1}). Two microlitres portion of each sample were injected in splitless mode. The injector was kept at 290 °C and the Transfer Line at 300 °C; the column oven was initially kept at a temperature of 90 °C for 1 min, and then heated up to 150 °C at a rate of 20 °C min^{-1}, and then to 300 °C at 4 °C min^{-1}, with post run heating at 315 °C (15 min). Analyses were performed in SIM mode using octachloronaphtalene (OCN) as procedural internal standard. Concentrations found were corrected using instrumental response factors.

3.5 Identification of bacteria isolates

HM-tolerant and PCB-oxidizing bacterial isolates were identified by the 16S rRNA gene sequencing. Single colonies of each strain were lysed by heating at 95 °C for 10 min. Amplification of 16S rRNA gene was performed with a thermocycler (Mastercycler GeneAmp PCR-System 9700, Applied Biosystem, USA) using bacteria-specific primers 27F (5′–AGAGTTTGATC(AC)TGGCTCAG–3′) and 1492R (5′–TACGGYTACCTTGTTACGAC-3′). The reaction mixtures were assembled at 0 °C and contained 2 µL DNA, 1 µL of each of the two primers (10 µM), 5 µL of reaction buffer 10×, 0.25 µL of Taq polymerase My Taq PRIME (5 U μL^{-1}), and sterile Milli-Q water to a final volume of 25 µL. Negative controls for DNA extraction and PCR setup (reaction mixture without a DNA template) were also used in every PCR run. The PCR program was as follows: 1) 95 °C for 1 min; 2) 35 cycles at 95 °C for 30 s, 52 °C for 30 s and 72 °C for 30 s 3) 72 °C for 10 min. Quality of DNA was checked by running 2 µL of each sample on 1% agarose gel (w/v) (Bioline) in TAE buffer 1X (0.04 M Tris-acetate, 0.02 M acetic acid, 0.001 M EDTA) (Fermentas) containing Syber Safe (1µL/25mL final concentration) (Invitrogen).

Amplified products were sequenced at the EurofinsEurope (Germany). Sequences were then read and corrected manually using the software FinchTV version 1.4. Next relatives of isolates were determined by comparison sequences in the NCBI GenBank and the EMBL databases using BLAST,

and the “Seqmatch” and “Classifier” programs of the Ribosomal Database Project II (http://rdp.cme.msu.edu/) (Altschul et al., 1997).

3.6 Phylogenetic analysis

Analyses were conducted using MEGA12 (Kumar et al., 2024), using the Maximum Likelihood method with the best-fitting nucleotide substitution model. The phylogenetic trees were rooted using *Pyrococcus furiosus* as an outgroup, providing a clear evolutionary reference for the inferred relationships among the studied strains.The percentage of replicate trees in which the associated taxa clustered together, where the number of replicates (100) (Kumar et al., 2024), is shown next to the branches. The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree (Saitou & Nei, 1987) and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the Tamura & Nei (1993) model. The MP tree had the shortest length among 10 MP tree searches, each performed with a randomly generated starting tree. The analytical procedure encompassed 45 coding nucleotide sequences using 1st, 2nd, 3rd, and non-coding positions with 1.581 positions in the final dataset.

4 Results and discussion

4.1 Prokaryotic community biodiversity

4.1.1 Arctic lakes

4.1.1.1 Solvannet Lake

Overall, the prokaryotic community in water and sediment samples collected from Solvannet Lake (L1) was composed of 346 and 1192 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 7.5% (Fig. 6a).

Water. A total of 80598 reads were detected, of which 71436 were of good quality (88.63%). The Shannon H index showed a value of 2.23, while the Evenness index was 0.027 (Tab. 2). Bacteroidota predominated, accounting for 55% of total sequences, followed by Gammaproteobacteria (36%) and Actinobacteriota (6%) (Fig. 7a). At the family level, an evident predominance of Flavobacteriaceae (53%; phylum Bacteroidota) was observed, followed by Burkholderiaceae and Comamonadaceae (18 and 17 %, respectively; both among Proteobacteria) (Fig 8a). Finally, at the genus level, *Flavobacterium* was the most abundant genus (53%), followed by *Polynucleobacter* (18%) and *Limnohabitans* (14%) (Fig. 9a).

Sediment. A total of 104422 reads were detected, out of which 89188 were of good quality (85.41%). The Shannon H index was 5.78, while the Evenness index was 0.273 (Tab. 2).
Gammaproteobacteria and Bacteroidota were equally predominant (31 and 29%, respectively), followed by Desulfobacterota and Alphaproteobacteria (both at 10%) (Fig. 7a). At the family level, the predominance of Bacteroidetes vadinHA17 (14%; phylum Bacteroidota) was observed, followed

by Comamonadaceae (12%) and Hydrogenophilaceae (8%), belonging to the Proteobacteria (Fig. 8a). In addition, 10% of detected families were unknown. Finally, at the genus level, most sequences were assigned to unknown genera (37%), followed by *Thiobacillus* (7%) (Fig. 9a).

4.1.1.2 Glacier Lake

Water. A total of 84935 reads were detected, out of which 75813 were of good quality (89.26%), for a total of 666 ASVs. The Shannon H index was 3.99, while the Evenness index was 0.081 (Tab. 2). Actinobacteriota and Gammaproteobacteria were the most abundant phyla (30 and 27% of total sequences, respectively), followed by Bacteroidota (21%) and Alphaproteobacteria (16%) (Fig. 7b). At the family level, Sporichthyaceae (21%; phylum Actinobacteriota), Comamonadaceae and Sphingomonadaceae (17 and 15%, respectively; phylum Proteobacteria) predominated (Fig 8b). At the genus level, the *hgcI clade* was the most abundant genus (19%), followed by *Sphingorhabdus* (14%) and *Polaromonas* (14%) (Fig 9b).

Sediment. No data are available for Glacier Lake sediments due to a low yield obtained for extracted DNA.

4.1.1.3 Knudsenheia Lake

Overall, the prokaryotic community in water and sediment samples collected for Knudsenheia Lake (L3) composed of 416 and 1260 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 7% (Fig. 6b).

Water. A total of 90336 reads were detected, out of which 78792 were of good quality (87.22%). The Shannon H index showed a value of 3.39, while the Evenness index indicated a value of 0.071 (Tab. 2).

Bacteroidota predominated, accounting for 44% of total sequences, followed by Actinobacteriota (32%) and Gammaproteobacteria (19%) (Fig. 7c). At the family level, Sporichthyaceae (16%; phyum Actinobacteriota), Crocinitomicaceae and Spirosomaceae (15 and 11%, respectively; phylum Bacteroidota) predominated (Fig. 8c). Finally, at the genus level, *hgcI clade* and *Fluviicola* were the most abundant genus (16 and 15%), followed by *Pseudarcicella* (11%) (Fig. 9c).

Sediment. A total of 103725 reads were detected, out of which 93322 were of good quality (89.97%). The Shannon H index showed a value 5.03, while the Evenness index indicated a value 0.122 (Tab. 2).

Bacteroidota was the most abundant Phyla (46%), followed by Desulfobacterota (16%) and Actinobacteriota (8%) (Fig. 7c). At the family level, the predominance of group Bacteroidetes vadinHA17 (34%; phylum Bacteroidota) was observed, followed by Desulfosarcinaceae (6%; phylum Campylobacterota) and Prolixibacteraceae (5%; phylum Bacteroidota) (Fig. 8c). In addition, 16% of sequence detected families were unknow. Finally, at the genus level, most sequences were assigned to unknown genera (55%) (Fig. 9c).

4.1.1.4 Storvatnet Lake

Overall, the prokaryotic community in water and sediment samples collected for Storvatnet Lake (L4) composed of 1255 and 1766 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 12.9% (Fig. 6c).

Water. A total of 106004 were detected, out of which 91905 were of good quality (86.70%). The Shannon H index showed a value of 4.91, while the Evenness index indicated a value of 0.108 (Tab. 2).

Gammaproteobacteria predominated, accounting for 34% of total sequences (Fig. 7d), followed by Actinobacteriota (26%) and Alphaproteobacteria (17%). At the family level, the predominance of Comamonadaceae (17%; phylum Proteobacteria) was observed, followed by Burkholderiaceae (10%; phylum Proteobacteria) and Mycobacteriaceae (7%; phylum Actinobacteriota) (Fig. 8d). Finally, at the genus level, *Polaromonas* and *Polynucleobacter* were the most abundant genus (10%), followed by *Mycobacterium* (7%). In addition, 20% of sequences detected were assigned to unknown genera (Fig. 9d)

Sediment. A total of 106172 reads were detected, out of which 90444 were of good quality (85.19%). The Shannon H index showed a value of 5.99, while the Evenness index indicated a value of 0.226 (Tab. 2). Actinobacteriota was the predominant phylum (29%), followed by Gammaproteobacteria (22%) and Desulfobacterota (11%) (Fig. 7d). At the family level, the predominance of Intrasporangiaceae (13%; phylum Actinobacteriota) was observed, followed by Hydrogenophilaceae (6%; phylum Proteobacteria) and Syntrophobacteraceae (5%; phylum Desulfobacterota) (Fig. 8d). In addition, 27% of detected families were unknow. Finally, at the genus level, most sequences were assigned to unknown genera (42%), followed by *Oryzihumus* (11%) (Fig. 9d).

4.1.1.5 Tvillingvatnet Lake

Overall, the prokaryotic community in water and sediment samples collected for Tvillingvatnet Lake (L5) was composed of 674 and 1874 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 8.1% (Fig. 6d).

Water. A total of 91448 reads were detected, out of which 83439 were of good quality (91.24%). The Shannon H index showed a value of 3.25, while the Evenness index indicated a value of 0.038 (Tab. 2).

Actinobacteriota was the predominant phylum (64%), followed by Gammaproteobacteria (22%) and Alphaproteobacteria (8%) (Fig. 7e). At the family level, the predominance of Sporichthyaceae (55%; phylum Actinobacteriota) was observed, followed by Burkholderiaceae (13%; phylum Proteobacteria) and unkown family (8%) (Fig. 8e). Finally, at the genus level, *hgcI clade* was the most abundant genus (48%), followed by *Polynucleobacter* (7%) and unknown genera (11%) were detected (Fig. 9e).

Sediment. A total of 102144 reads were detected, out of which 83173 were of good quality (81.43%). The Shannon H index showed a value 6.55, while the Evenness index indicated a value 0.373 (Tab. 2).

Actinobacteriota was the predominant phylum (33%), followed by Gammaproteobacteria (15%) and Bacteroidota and Alphaproteobacteria (12 and 10%, respectively) (Fig. 7e). At the family level, the predominance of unknown family (27%) was observed, followed by Intrasporangiaceae (7%; phylum Actinobacteriota) and Bacteroidetes vadinHA17 (5%; phylum Bacteroidota) (Fig. 8e). Finally, at the genus level the majority of sequences were assigned to unknown genera (48%), followed by *Oryzihumus* (7%) and *Gaiella* (4%) (Fig. 9e).

4.1.1.5 Diversity and distribution of prokaryotes in Arctic lakes

In general, the prokaryotic community in water and sediment samples collected from all Arctic lakes was composed of 2506 and 4861 ASVs respectively. Based in the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices and the detected ASVs showed an overlap of 10.5% (Fig. 10a), In particular, the prokaryotic community in water ranged to a minimum of 346 ASVs in Solvannet Lake (L1), to a maximum to 1255 ASVs in Knudsenheia Lake

(L3). Instead, in sediment ranged to a minimum of 1260 ASVs in Storvatnet Lake (L4) to a maximum to 1874 ASVs in Tvillingvatnet (L5). The overlap about the two matrices ranged to a minimum to 7% in Knudsenheia Lake (L3) to a maximum to 12.9% in Storvatnet Lakes (L4).

A total of 869784 reads were found in the Arctic lakes of which 757512 were of good quality (87.23%). The Shannon H diversity index values ranged from a minimum of 2.23 (water of Solvannet Lake, L1) to a maximum of 6.55 (sediment of Tvillingvatnet Lake, L5), while the Evenness index values ranged from a minimum of 0.027 (water of Solvannet Lake, L1) to a maximum of 0.373 (sediment of Tvillingvatnet Lake, L5).

In general, water communities were mainly composed of Actinobacteriota, Bacteroidota, Alphaproteobacteria, and Gammaproteobacteria. In sediments, in addition to these phyla, Acidobacteriota, Desulfobacterota, and Firmicutes were also present.

Across lakes, Actinobacteriota, Bacteroidota, and Gammaproteobacteria were consistently dominant phyla, though their relative abundances varied between lakes and matrices. In water samples, Actinobacteriota were highly abundant in Tvillingvatnet Lake (L5) (64%) and Glacier Lake (L2) (30%), while Bacteroidota predominated in Solvannet Lake (L1) (55%) and Knudsenheia (L3) (44%). In sediments, Bacteroidota and Gammaproteobacteria were co-dominant in Solvannet Lake (L1) (29% and 31%, respectively), while Knudsenheia Lake (L3) had a substantial presence of Bacteroidota (46%) and Desulfobacterota (16%). Sediments also exhibited higher proportions of Acidobacteriota, such as in Storvatnet Lake (L4) (9%) and Tvillingvatnet Lake (L5) (12%), a phylum almost absent in water samples.

Across lakes, Actinobacteriota, Bacteroidota, and Gammaproteobacteria were consistently dominant phyla, though their relative abundances varied between lakes and matrices. In water samples, Actinobacteriota were highly abundant, especially in Tvillingvatnet Lake (L5) (64%) but also in Knudsenheia Lake (L3) (32%), Glacier Lake (L2) (30%) and Storvatnet (L4) (26%). In comparison, Bacteroidota predominated in Solvannet Lake (L1) (55%) and Knudsenheia (L3) (44%) and also in

Glacier Lake (L2) (21%) and Storvatnet (L4) (11%). Finally, Gammaproteobacteria were highly abundant in all the lakes, mainly in Solvannet Lake (L1) (36%) and Storvatnet Lake (L4) (34%). Water also exhibited higher proportions of Alphaproteobacteria in the Storvatnet (L4) and Glacier (L2) Lakes (both at 17%).

In sediments, Bacteroidota and Gammaproteobacteria were co-dominant in Solvannet Lake (L1) (29% and 31%, respectively), while Knudsenheia Lake (L3) had a substantial presence of Bacteroidota (46%) and Desulfobacterota (16%). At the same time, the Storvatnet (L4) and Tvillingvatnet Lakes (L5) have a high abundance of Actinobacteriota (29 and 33%, respectively) and Gammaproteobacteria (22 and 15%). Finally, Desulfobacterota also exhibited a higher abundance in Storvatnet (L4) (11%) and Solvannet Lakes (L1) (10%), a phylum almost absent in water samples.

At the family level, water communities varied significantly between lakes. Sporichthyaceae dominated the water of Tvillingvatnet Lake (L5) (55%) and Glacier Lake (L2) (21%), while Flavobacteriaceae were predominant in Solvannet Lake (L1) (53%). Comamonadaceae exhibited higher abundance in Solvannet (L1), Storvatnet (L4), and Glacier Lakes (L2) (all at 17%).

In contrast, sediment communities featured families such as Bacteroidetes vadinHA17, which were abundant in Knudsenheia Lake (L3) (34%) and Solvannet Lake (L1) (14%). Sediments also contained substantial unknown families, particularly in Storvatnet Lake (L4) (27%) and Tvillingvatnet Lake (L5) (27%), reflecting unexplored microbial diversity.

Differences were also notable at the genus level. The *hgcI clade* was dominant in the water of Tvillingvatnet Lake (L5) (48%) and Glacier Lake (L2) (19%), while *Flavobacterium* prevailed in Solvannet's Lake water (L1) (53%). Sediments were characterized by unknown genera, which accounted for 37% of sequences in Solvannet Lake (L1), 55% in Knudsenheia Lake (L3), and 42% in Storvatnet Lake (L4), highlighting the ecological uniqueness of Arctic lake sediments. Notable known genera in sediments included *Thiobacillus* in Solvannet Lake (L1) (7%) and *Oryzihumus* in Storvatnet Lake (L4) (11%).

From the nMDS graph constructed with ASVs data, it can be observed that the ASVs from the water and sediments of the various lakes were significantly different from each other (Fig. 11a). Moreover, the water communities of Tvillingvatnet (L5), Glacier (L2), and Knudsenheia (L3) were closer to each other compared to the Storvatnet (L4) and Solvannet lakes (L1). On the other hand, the most distinct communities were those of Solvannet (L1) and Tvillingvatnet (L5). Regarding the sediments, it was found that the communities in Storvatnet (L4) and Tvillingvatnet (L5) were more similar compared to the other lakes.

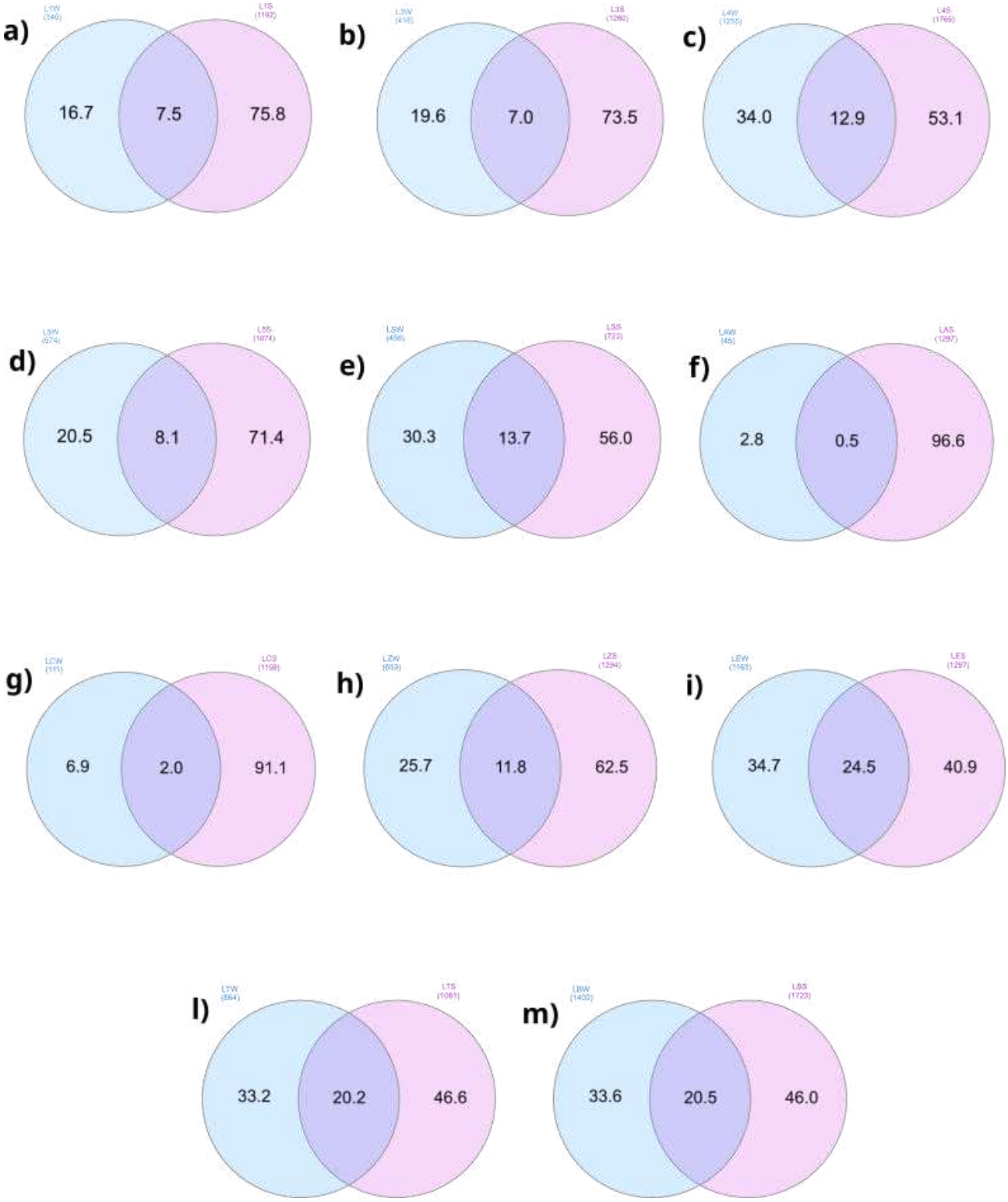


Fig. 6: Venn diagrams constructed on the total retrieved ASVs and separated for each lake. Water and sediment samples were shown in Sky Blue and lilac, respectively. Arctic lakes are as follows: a) Solvannet Lake (L1); b) Knudsenheia Lake (L3); c) Storvatnet Lake (L4); d) Tvillingvatnet Lake (L5). Antarctic lakes are as follows: e) Sofia Lake (LS); f) Argentina Lake (LA); g) Crater Lake (LC); h) Zapatilla Lake (LZ); i) Extremadura Lake (LE); l) Telefon Lake (LT); m) Ballaneros Lake (LB). W: water sample; S: sediment sample.

Tab. 2: Sequence data and diversity indices

	Lake	Samples	Reads in	Good quality reads	Good quality reads %	Shannon *H*	Evenness e^H/S	Simpson	InvSimpson	Fisher	Chao1
Arctic	Solvannet	L1W	80598	71436	88.63	2.233	0.027	0.661	2.951	10.799	96.909
		L1S	104422	89188	85.41	5.784	0.273	0.814	5.388	24.097	201.111
	Glacier	L2W	84935	75813	89.26	3.990	0.081	0.904	10.450	19.062	159.615
		L2S									
	Knudsenheia	L3W	90336	78792	87.22	3.390	0.071	0.903	10.358	13.100	116.333
		L3S	103725	93322	89.97	5.034	0.122	0.593	2.457	26.580	217.188
	Storvatnet	L4W	106004	91905	86.70	4.905	0.108	0.918	12.121	30.594	250.500
		L4S	106172	90444	85.19	5.990	0.226	0.727	3.660	26.837	219.909
	Tvillingvatnet	L5W	91448	83439	91.24	3.246	0.038	0.736	3.794	17.470	149.250
		L5S	102144	83173	81.43	6.549	0.373	0.670	3.034	27.157	218.500
Antarctica	Sofia	LSW	111664	104220	93.33	3.535	0.047	0.848	6.581	18.922	163.273
		LSS	100640	89071	88.50	4.596	0.217	0.790	4.762	12.260	110.111
	Argentina	LAW	81574	77290	94.75	1.003	0.061	0.348	1.535	1.987	22.000
		LAS	109229	96470	88.32	5.882	0.277	0.910	11.085	24.799	205.273
	Crater	LCW	86715	81738	94.26	1.904	0.060	0.775	4.446	5.172	54.200
		LCS	82434	74163	89.97	5.840	0.297	0.742	3.869	22.462	182.000
	Zapatilla	LZW	109606	101215	92.34	3.774	0.067	0.890	9.106	19.260	165.063
		LZS	91928	81872	89.06	5.620	0.213	0.866	7.437	24.809	201.333
	Extremadura	LEW	89800	78413	87.32	5.148	0.148	0.889	9.014	29.845	237.400
		LES	127978	108108	84.47	5.313	0.158	0.877	8.163	22.000	190.000
	Telefon	LTW	75430	67983	90.13	4.455	0.100	0.879	8.294	25.622	203.000
		LTS	100950	89802	88.96	5.122	0.155	0.881	8.428	28.133	229.154
	Ballaneros	LBW	94768	83246	87.84	4.975	0.103	0.899	9.944	38.103	295.333
		LBS	119498	100701	84.27	6.001	0.234	0.881	8.420	28.660	245.667

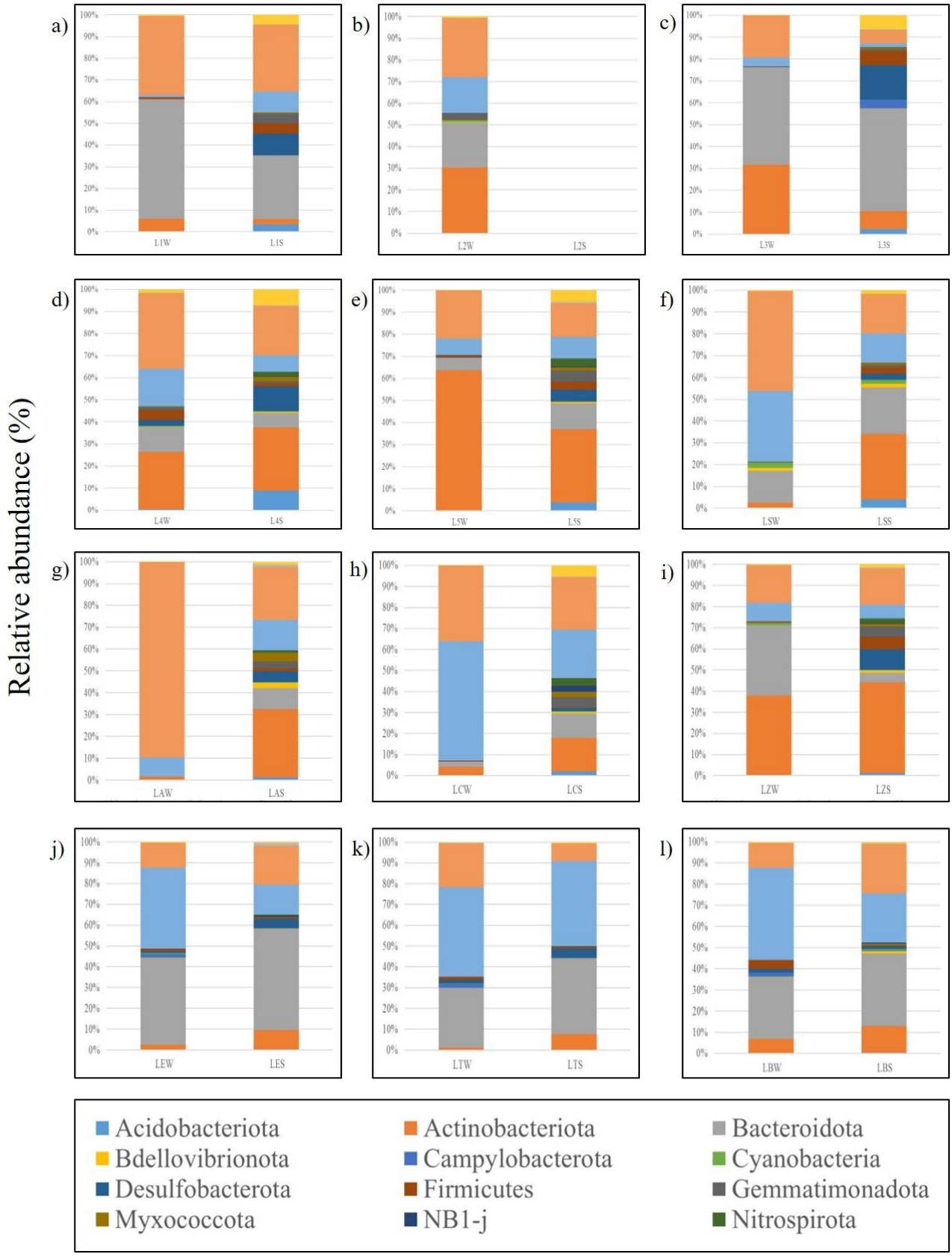


Fig. 7: Composition of the prokaryotic community at phylum level (>2 %). Arctic lakes are as follows: a) Solvannet Lake (L1); b) Glacier Lake (L2); c) Knudsenheia Lake (L3); d) Storvatnet Lake (L4); e) Tvillingvatnet Lake (L5). Antarctic lakes are as follows: f) Sofia Lake (LS); g) Argentina Lake (LA); h) Crater Lake (LC); i) Zapatilla Lake (LZ); j) Extremadura Lake (LE); k) Telefon Lake (LT); l) Ballaneros Lake (LB). W: water sample; S: sediment sample.

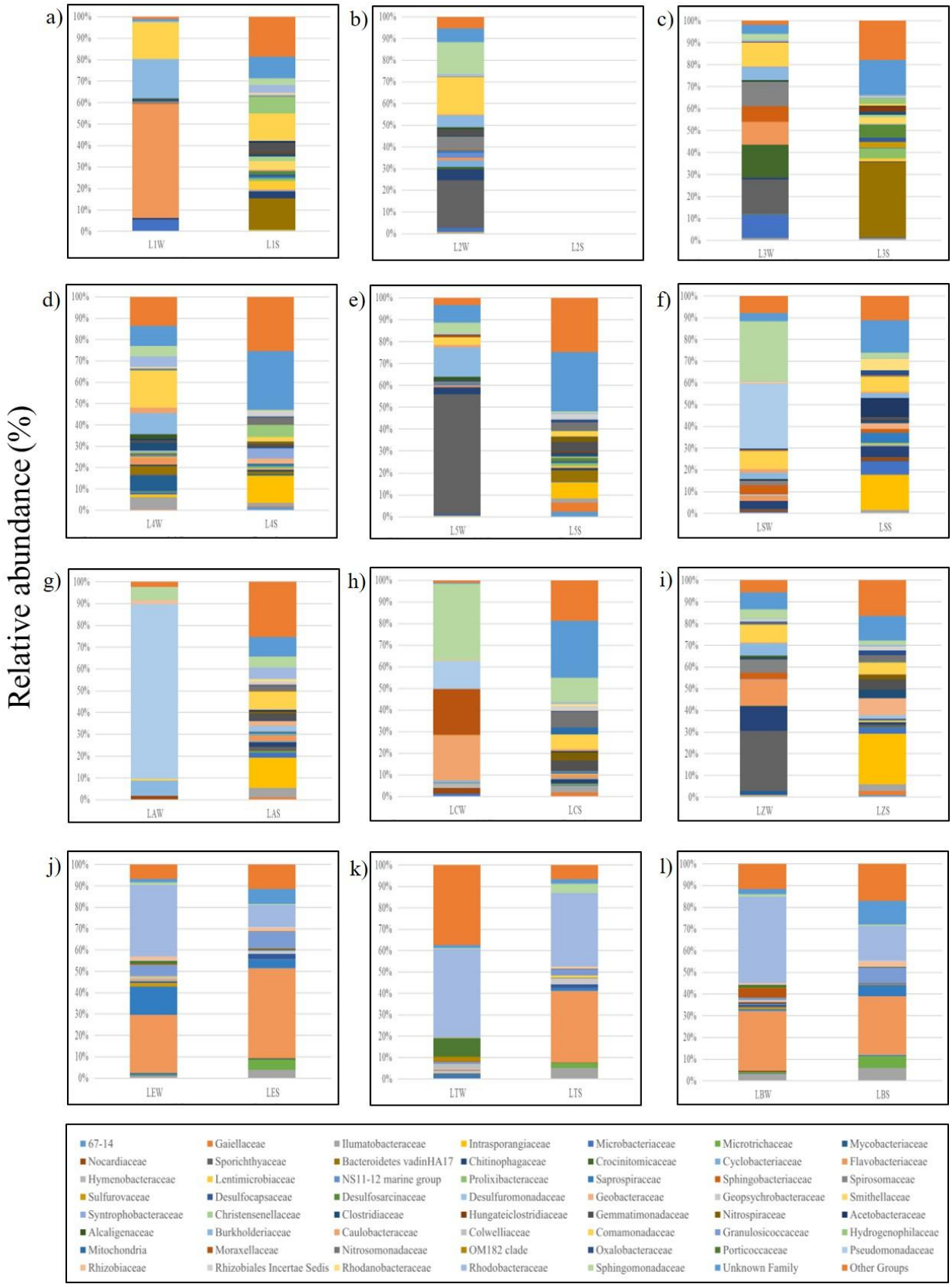


Fig. 8: Composition of the prokaryotic community at family level (>2 %). Arctic lakes are as follows: a) Solvannet Lake (L1); b) Glacier Lake (L2); c) Knudsenheia Lake (L3); d) Storvatnet Lake (L4); e) Tvillingvatnet Lake (L5). Antarctic lakes are as follows: f) Sofia Lake (LS); g) Argentina Lake (LA); h) Crater Lake (LC); i) Zapatilla Lake (LZ); j) extremadura Lake (LE); k) Telefon Lake (LT); l) Ballaneros Lake (LB). W: water sample; S: sediment sample.

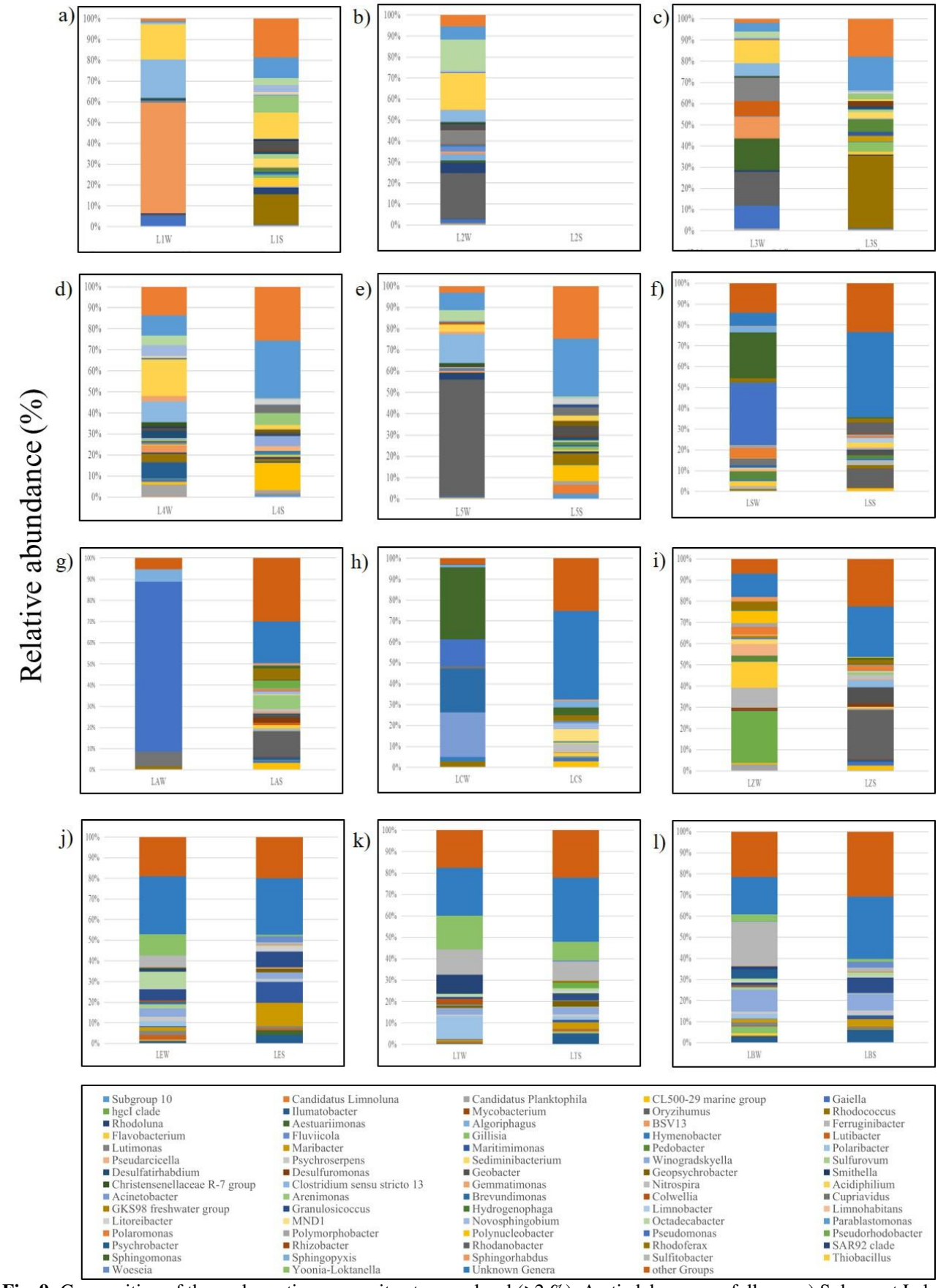


Fig. 9: Composition of the prokaryotic community at genus level (>2 %). Arctic lakes are as follows: a) Solvannet Lake (L1); b) Glacier Lake (L2); c) Knudsenheia Lake (L3); d) Storvatnet Lake (L4); e) Tvillingvatnet Lake (L5). Antarctic lakes are as follows: f) Sofia Lake (LS); g) Argentina Lake (LA); h) Crater Lake (LC); i) Zapatilla Lake (LZ); j) Extremadura Lake (LE); k) Telefon Lake (LT); l) Ballaneros Lake (LB). W: water sample; S: sediment sample.

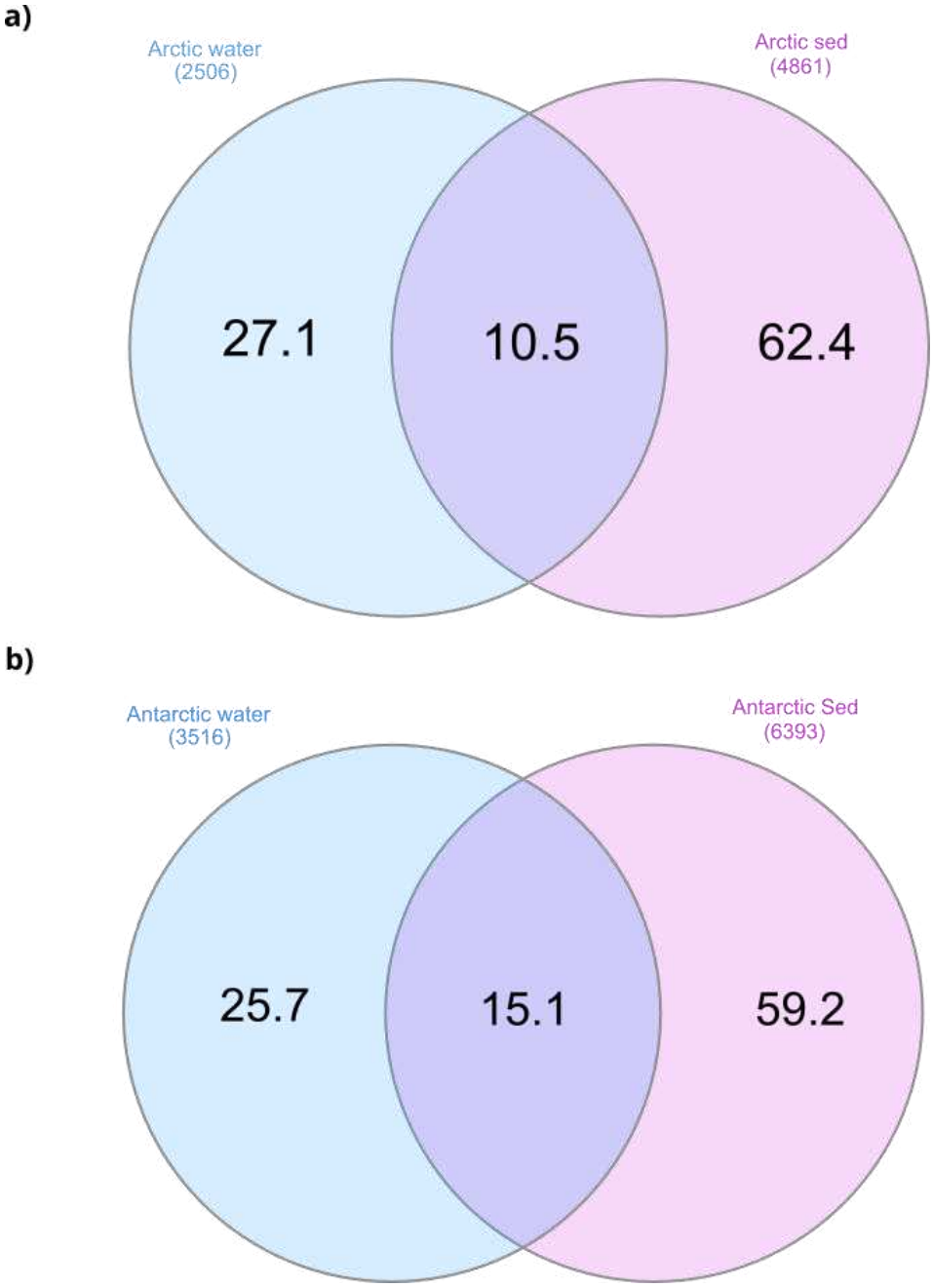


Fig. 10: Venn diagrams constructed on the total retrieved ASVs and separated in Arctic and Antarctic for water and sediments samples. Water and sediment samples were in Sky Blue and lilac, respectively.

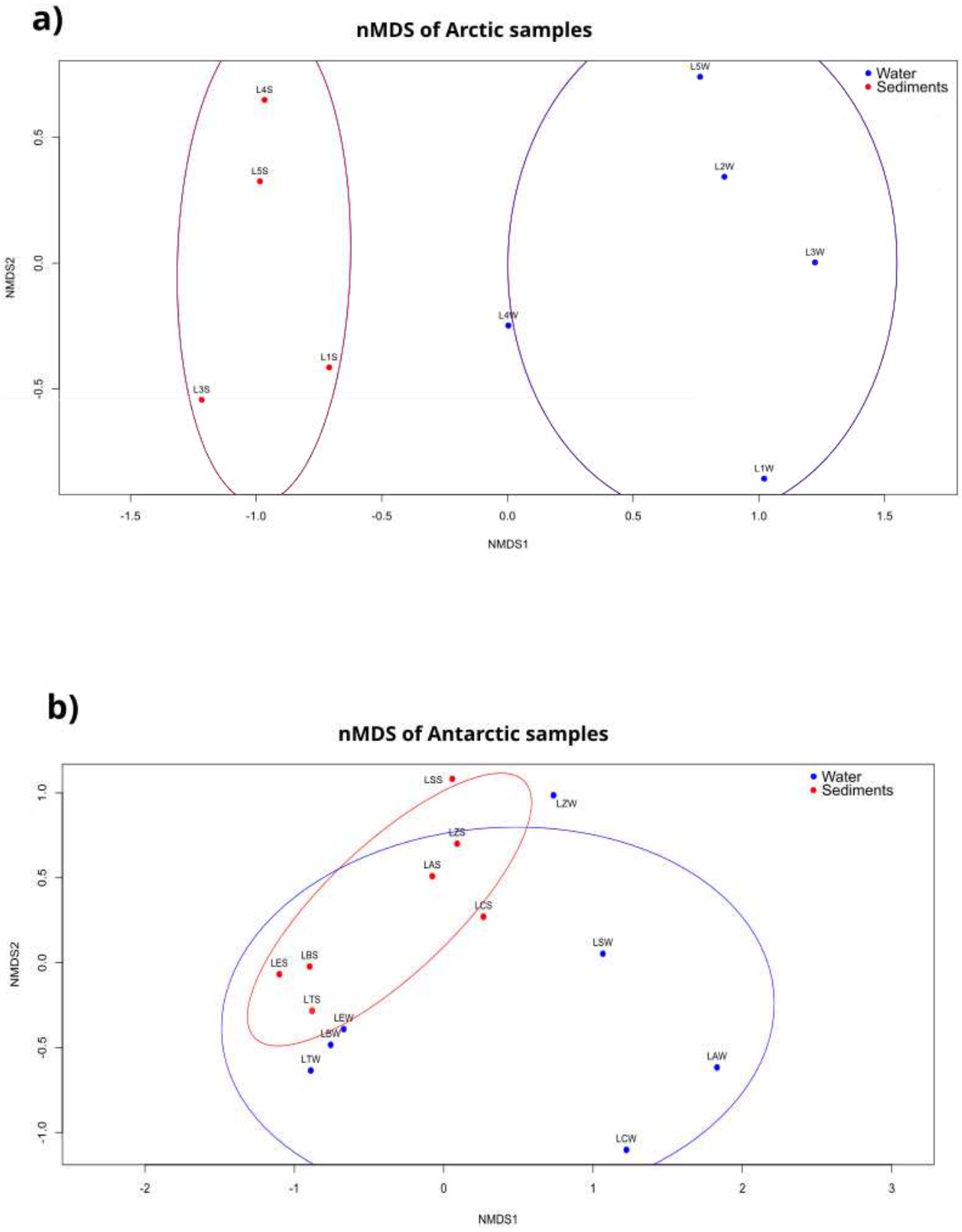


Fig. 11: nMDS of a) Arctic and b) Antarctic samples.

4.1.2 Antarctic lakes

4.1.2.1 Sofia Lake

Overall, the prokaryotic community in water and sediment samples collected for Sofia Lake (LS) was composed of 456 and 723 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 13.7% (Fig. 6e).

Water. A total of 111664 reads were detected, out of which 104220 were of good quality (83.3%). The Shannon H index showed a value of 3.54, while the Evenness index indicated a value of 0.047 (Tab. 2).

Gammaproteobacteria predominated, accounting for 46% of total sequences (Fig. 7f), followed by Alphaproteobacteria (32%) and Bacteroidota (15%). At the family level, Pseudomonadaceae and Sphingomonadaceae were equally predominant (30 and 28 %; both among Proteobacteria), followed by Comamonadaceae (8%; phylum Proteobacteria) (Fig. 8f). Finally, at the genus level, *Pseudomonas* was the most abundant genus (30%), followed by *Sphingomonas* (22%) and *Polaromonas* (5%) (Fig. 9f).

Sediment. A total of 100640 merged reads were detected, out of which 89071 were of good quality (88.50%). The Shannon H index showed a value 4.60, while the Evenness index indicated a value 0.217 (Tab. 2).

Actinobacteriota was the most abundant Phyla (30%), followed by Bacteroidota (21%) and Gammaproteobacteria (18%) (Fig. 7f). At the family level, the predominance of Intrasporangiaceae (16%; phylum Actinobacteriota) was observed, followed by Acetobacteraceae (9%; phylum Proteobacteria) and Comamonadaceae (7%; phylum Proteobacteria) (Fig. 8f). In addition, 15% of detected families were unknow.

Finally, at the genus level the majority of sequences were assigned to unknown genera (41%), followed by *Oryzihumus* (9%) (Fig. 9f).

4.1.2.2 Argentina Lake

Overall, the prokaryotic community in water and sediment samples collected for Argentina Lake (LA) was composed of 45 and 1297 ASVs respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 0.5% (Fig. 6f).

Water. A total of 81574 merged reads were detected, out of which 77290 were of good quality (94.75%). The Shannon H index showed a value of 1.00, while the Evenness index indicated a value of 0.061 (Tab. 2).

Gammaproteobacteria predominated, accounting for 90% of total sequences, followed by Alphaproteobacteria (9%) (Fig. 7g). At the family level, the predominance of Pseudomonadaceae (80%; phylum Proteobacteria) was observed, followed by Burkholderiaceae and Sphingomonadaceae (7 and 6%; both among Proteobacteria) (Fig. 8g). Finally, at the genus level, *Pseudomonas* was the most abundant genus (80%), followed by *Cupriavidus* (7%) and *Sphingopyxis* (6%) (Fig. 9g).

Sediment. A total of 109229 reads were detected, out of which 96470 were of good quality (88.32%). The Shannon H index showed a value 5.88, while the Evenness index indicated a value 0.277 (Tab. 2).

Actinobacteriota was the most abundant Phyla (31%), followed by Gammaproteobacteria (24%) and Alphaproteobacteria (14%) (Fig. 7g). At the family level, the predominance of Intrasporangiaceae (14%; phylum Actinobacteriota) was observed, followed by Comamonadaceae (8%; phylum Proteobacteria), Rhodobacteraceae and Sphingomonadaceae (both at 5%; both among phylum Proteobacteria) (Fig. 8g). In addition, 19% of detected families were unknow.

Finally, at the genus level the majority of sequences were assigned to unknown genera (19%), followed by *Oryzihumus* (12%) (Fig. 9g).

4.1.2.3 Crater Lake

Overall, the prokaryotic community in water and sediment samples collected for all Crater Lake (LC) composed of 111 and 1159 ASVs respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 2% (Fig. 6g).

Water. A total of 86715 reads were detected, out of which 81738 were of good quality (94.26%). The Shannon H index showed a value of 1.90, while the Evenness index indicated a value of 0.060 (Tab. 2).

Alphaproteobacteria predominated, accounting for 57% of total sequences, followed by Gammaproteobacteria (36%) (Fig. 7h). At the family level, the predominance of Sphingomonadaceae (35%; phylum Proteobacteria) was observed, followed by Moraxellaceae and Caulobacteraceae (both at 21%; both among phylum Proteobacteria) and Pseudomonadaceae (13%; phylum Proteobacteria) (Fig. 8h).

Finally, at the genus level, *Sphingomonas* was the most abundant genus (34%), followed by *Acinetobacter* and *Brevundimonas* (both at 21%) and *Pseudomonas* (13%) (Fig. 9h).

Sediment. A total of 82434 reads were detected, out of which 74163 were of good quality (89.97%). The Shannon H index showed a value 5.84, while the Evenness index indicated a value 0.297 (Tab. 2).

Gammaproteobacteria (25%) and Alphaproteobacteria (23%) were the most abundant phyla, followed by Actinobacteriota (16%) and Bacteroidota (12%) (Fig 7h). At the family level, the predominance of Sphingomonadaceae (11%; phylum Proteobacteria) was observed, followed by

Nitrosomonadaceae and Comamonadaceae (both at 7%; both among Proteobacteria) (Fig. 8h). In addition, 26% of detected families were unknow.

Finally, at the genus level most sequences were assigned to unknown genera (42%), followed by *MND1* (6%) (Fig. 9h).

4.1.2.4 Zapatilla Lake

Overall, the prokaryotic community in water and sediment samples collected for all Zapatilla Lake (LZ) was composed of 653 and 1294 ASVs, respectively. Base on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 11.8% (Fig. 6h).

Water. A total of 109606 reads were detected, out of which 101215 were of good quality (92.34 %). The Shannon H index showed a value of 3.77, while the Evenness index indicated a value of 0.067 (Tab. 2). Actinobacteriota (37%) and Bacteroidota (33%) were the most abundant phyla, followed by Gammaproteobacteria (18%) (Fig. 7i). At the family level, the predominance of Sporichthyaceae (28%; phylum Actinobacteriota) was observed, followed by Flavobacteriaceae and Chitinophagaceae (12 and 11%; both among phylum Bacteroidota) (Fig 8i). Finally, at the genus level, *hgcI clade* was the most abundant genus (25%), followed by *Flavobacterium* (12%) and *Ferruginibacter* (9%) (Fig 9i). In addition, 11% of detected genus were unknow.

Sediment. A total of 91928 reads were detected, out of which 81872 were of good quality (89.06%). The Shannon H index showed a value 5.62, while the Evenness index indicated a value 0.213 (Tab. 2). Actinobacteriota was the most abundant phyla (43%), followed by Gammaproteobacteria (17%) and Desulfobacterota (10%) (Fig. 7i). At the family level, the predominance of Intrasporangiaceae (23%; phylum Actinobacteriota) was observed, followed by Geobacteraceae (8%; phylum Desulfobacterota), Comamonadaceae and Gemmatimonadaceae (both at 5%; phyla Proteobacteria

and Gemmatimonadota respectively) (Fig. 8i). In addition, 11% of detected families were unknow. Finally, at the genus level, most sequences were assigned to unknown genera (24%), followed by *Oryzihumus* with similar abundance (23%) and *Geobacter* (8%) (Fig. 9i).

4.1.2.5 Extremadura Lake

Overall, the prokaryotic community in water and sediment samples collected for Extremadura Lake (LE) was composed of 1165 and 1287 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 24.5% (Fig. 6i).

Water. A total of 89800 merged reads were detected, out of which 78413 were of good quality (87.32%). The Shannon H index showed a value of 5.15, while the Evenness index indicated a value of 0.148 (Tab. 2).

Bacteroidota (41%) and Alphaproteobacteria (39%) were the most abundant phylum, followed by Gammaproteobacteria (12%) (Fig. 7j). At the family level, the predominance of Rhodobacteraceae (34%; phylum Proteobacteria) was observed, followed by Flavobacteriaceae and Saprospiraceae (27 and 13%; both among phylum Bacteroidota) (Fig. 8j). Finally, at the genus level the majority of sequences were assigned to unknown genera (28%), followed by *Yoonia-Loktanella* (10%) and *Octadecabacter* (8%) (Fig. 9j).

Sediment. A total of 127978 reads were detected, out of which 108108 were of good quality (84.47%). The Shannon H index showed a value 5.31, while the Evenness index indicated a value 0.158 (Tab. 2).

Bacteroidota was the most abundant Phyla (48%), followed by Gammaproteobacteria (19%) and Alphaproteobacteria (15%) (Fig. 7j). At the family level, the predominance of Flavobacteriaceae

(42%; phylum Bacteroidota) was observed, followed by Rhodobacteraceae and Granulosicoccaceae (10 and 8%; both among phylum Proteobacteria) (Fig. 8j). Finally, at the genus level the majority of sequences were assigned to unknown genera (27%), followed by *Maribacter* (11%) and *Maritimimonas* (10%) (Fig. 9j).

4.1.2.6 Telefon Lake

Overall, the prokaryotic community in water and sediment samples collected for Telefon Lake (LT) was composed of 864 and 1081 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 20.2% (Fig. 6l).

Water. A total of 75430 reads were detected, out of which 67983 were of good quality (90.13%). The Shannon H index showed a value of 4.46, while the Evenness index indicated a value of 0.100 (Tab. 2). Alphaproteobacteria (43%) was the most abundant phyla, followed by Bacteroidota (29%) and Gammaproteobacteria (21%) (Fig. 7k). At the family level, the predominance of Rhodobacteraceae (41%; phylum Proteobacteria) was observed, followed by Porticoccaceae (8%; phylum Proteobacteria) (Fig. 8k).

Finally, at the genus level, *Yoonia-Loktanella* was the most abundant genus (16%), followed by *Sulfitobacter* (12%) and *Polaribacter* (11%) (Fig. 9k). In addition, 22% of detected genus were unknown.

Sediment. A total of 100950 reads were detected, out of which 89802 were of good quality (88.96%). The Shannon H index showed a value 5.12, while the Evenness index indicated a value 0.155 (Tab. 2). Alphaproteobacteria was the most abundant Phyla (41%), followed by Bacteroidota (36%), Gammaproteobacteria (9%) and Actinobacteriota (8%) (Fig. 7k). At the family level, Rhodobacteraceae and Flavobacteriaceae were equally predominant (35 and 33%; phyla

Proteobacteria and Bacteroidota respectively), followed by Ilumatobacteraceae (5%; phylum Actinobacteriota) and Sphingomonadaceae (4%; phylum Proteobacteria) (Fig. 8k). Finally, at the genus level, the majority of sequences were assigned to unknown genera (30%), followed by *Yoonia-Loktanella* and *Sulfitobacter* (both at 9%) and *Ilumatobacter* (5%) (Fig. 9k).

4.1.2.7 Ballaneros Lake

Overall, the prokaryotic community in water and sediment samples collected for Ballaneros Lake (LB) was composed of 1402 and 1723 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 20.5% (Fig. 6m).

Water. A total of 94768 reads were detected, out of which 83246 were of good quality (87.84%). The Shannon H index showed a value of 4.98, while the Evenness index indicated a value of 0.103 (Tab. 2). Alphaproteobacteria (44%) was the most abundant phyla, followed by Bacteroidota (29%) and Gammaproteobacteria (11%) (Fig. 7l). At the family level, the predominance of Rhodobacteraceae (40%; phylum Proteobacteria) was observed, followed by Flavobacteriaceae (27%; phylum Bacteroidota) (Fig. 8l). Finally, at the genus level, *Sulfitobacter* was the most abundant genus (21%), followed by *Winogradskyella* (10%) (Fig. 9l). In addition, 18% of detected genus were unknown.

Sediment. A total of 119498 reads were detected, out of which 100701 were of good quality (84.27%). The Shannon H index showed a value 6.00, while the Evenness index indicated a value 0.234 (Tab. 2). Bacteroidota was the most abundant Phyla (34%), followed by Alphaproteobacteria and Gammaproteobacteria (both at 23%) and Actinobacteriota (12%) (Fig. 7m). At the family level, the predominance of Flavobacteriaceae was the equally predominant (27%; phylum Bacteroidota) was observed, followed by Rhodobacteraceae (16%; phylum Proteobacteria) (Fig. 8m). Finally, at the

genus level, the majority of sequences were assigned to unknown genera (29%), followed by *Winogradskyella* (8%) and *Granulosicoccus* (7%) (Fig. 9m).

4.1.2.8 Diversity and distribution of prokaryotes in Antarctic lakes

In general, the prokaryotic community in water and sediment samples collected for all Antarctic lakes was composed of 3516 and 6393 ASVs, respectively. Based on the Venn diagrams, which were constructed to identify ASVs shared between the two analyzed matrices, the detected ASVs showed an overlap of 15.1% (Fig. 10b). In particular, the prokaryotic community in water ranged to a minimum of 45 ASVs in Argentina Lake (LA), to a maximum to 1402 ASVs in Ballaneros Lake (LB). Instead, in sediment ranged to a minimum of 723 ASVs in Lake Sofia (LS) to a maximum to 1723 ASVs in Ballaneros Lake (LB). The overlap between the two matrices ranged from a minimum of 0.5% in Argentina Lake (LA) to a maximum of 24.5% in Extremadura Lake (LE). A total of 1382214 reads were found in the Arctic lakes of which 1234292 were of good quality (89.54%). The Shannon H diversity index values ranged from a minimum of 1 (water of Argentina Lake, LS) to a maximum of 6.55 (sediment of Ballaneros Lake, LB), while the Evenness index values ranged from a minimum of 0.047 (water of Sofia Lake, LS) to a maximum of 0.297 (sediment of Crater Lake, LC).

Across lakes, Bacteroidota, Alphaproteobacteria and Gammaproteobacteria were consistently dominant phyla, though their relative abundances varied between lakes and matrices. In water samples, in the Extremadura (LE), Telefon (LT) and Ballaneros Lakes (LB) showed the predominance of Bacteroidota (41, 29 and 29, respectively) and Alphaproteobacteria (39, 43 and 44 %, respectively). Alphaproteobacteria showed the presence in Crater (LC) (57%) and Sofia Lakes (LS) (32%); instead, Gammaproteobacteria were highly abundant, especially in Argentina (LA) (90%) and Sofia Lakes (LS) (46%). Actinobacteriota co-dominate with Bacteroidota in Zapatilla Lake (LZ) (38 and 33%, respectively).

In contrast, in sediment, Bacteroidota showed high abundance in the Extremadura (LE) (48%), Telefon (LT) (36%) and Ballaneros Lakes (LB) (34%). Instead, Alphaproteobacteria were highly abundant, especially in Telefon (LT) (41%), Ballaneros (LB) (23%) and Crater Lakes (LC) (23%). Gammaproteobacteria exhibited higher abundance in Crater (LC) (25%), Argentina (LA) (24%) and Ballaneros Lakes (LB) (23%). Sediment also exhibited higher proportions of Actinobacteriota in Zapatilla (LZ) (43%), Argentina (LA) (31%) and Sofia Lakes (LS) (30%).

At the family level, distinct patterns of distribution were observed between water and sediment communities in Antarctic lakes. *Intrasporangiaceae* were exclusively found in sediments, with notable abundances in Sofia Lake (LS) (16%), Argentina Lake (LA) (14%), and Zapatilla Lake (LZ) (23%), but were completely absent in the water of these lakes. In contrast, *Pseudomonadaceae* were present only in water samples, with high abundances in Sofia (LS) (30%), Argentina (LA) (80%), and Crater Lakes (LC) (13%), while being absent in their sediments. Brackish lakes, such as Extremadura (LE), Telefon (LT), and Ballaneros (LB), exhibited high similarity driven by families like *Flavobacteriaceae* and *Rhodobacteraceae*. *Flavobacteriaceae* were abundant in the water of Extremadura (LE) (27%), Ballaneros (LB) (27%), and Zapatilla Lakes (LZ) (12%), as well as in the sediments of Extremadura (LE) (42%), Telefon (LT) (33%), and Ballaneros Lakes (LB) (27%). Similarly, *Rhodobacteraceae* were detected in both water and sediment samples, with high abundances in the water of Extremadura (LE) (34%), Telefon (LT) (41%), and Ballaneros Lakes (LB) (40%) and the sediments of Extremadura (LE) (10%), Telefon (LT) (35%), and Ballaneros Lakes (LB) (16%).

Other families were detected across multiple lakes. *Comamonadaceae*were found in the water of Sofia (LS) and Zapatilla Lakes (LZ) (8% in both) and in the sediments of Sofia (LS) (7%), Argentina (LA) (8%), Crater (LC) (7%), and Zapatilla Lakes (LZ) (5%). *Sphingomonadaceae* were present in the water of Sofia (LS) (28%), Argentina (LA) (6%), Crater (LC) (35%), and Zapatilla Lakes (LZ) (4%) and in the sediments of Sofia (LS) (3%), Argentina (LA) (5%), and Crater Lakes (LC) (11%).

At the genus level, *Oryzihumus* was exclusively detected in sediments, with notable proportions in Sofia (LS) (9%), Argentina (LA) (12%), and Zapatilla Lakes (LZ) (23%). Conversely, *Pseudomonas* was restricted to water samples, with abundances in Sofia (LS) (30%), Argentina (LA) (80%), and Crater Lakes (LC) (13%). Similarly, *Sphingomonas* was predominantly found in water samples, particularly in Sofia (LS) (22%) and Crater Lakes (LC) (34%). Among brackish lakes, *Sulfitobacter* was detected in the water of Extremadura (LE) (6%), Telefon (LT) (12%), and Ballaneros Lakes (LB) (21%) and in the sediments of Telefon Lake (LT). Likewise, *Yoonia-Loktanella* was present in the water of Extremadura (LE) (10%) and Telefon Lakes (LT) (16%) and in the sediments of Telefon Lake (LT) (9%).

From the nMDS graph constructed with ASVs data, it can be observed that the ASVs between the sediments and the water partially overlapped by the ellipse constructed at 75% of similarity (Fig. 11b), but despite that, they showed notable separation. Moreover, it can be noted that the communities, both in the water and sediment, of lakes with brackish characteristics—Extremadura (LE), Telefon (LT), and Ballaneros (LB)—were similar to each other compared to the other lakes. In contrast, the water and sediments of the Zapadilla (LZ), Argentina (LA), and Crater Lakes (LC) were less similar to each other.

4.1.3 Diversity and distribution of prokaryotes in polar lakes

At the phylum level, there are no differences between the two poles. Four phyla were predominantly found in lakes, both in water and in sediments: Actinobacteriota, Bacteroidota, Alphaproteobacteria, and Gammaproteobacteria. However, Desulfobacterota were found in sediments at both poles, while Acidobacteriota were more prevalent in Arctic sediments.

At the family level, only a few families were exclusive to one pole. In the Arctic, Bacteroidetes vadinHA17 was present in both water and sediments, Crocinitomicaceae only in water, and Desulfosarcinaceae and Hydrogenophilaceae exclusively in sediments. In contrast, in Antarctica, Saprospiraceae was found in both water and sediment, while Moraxellaceae and Pseudomonadaceae

appeared only in water and Acetobacteraceae only in sediments. Families shared between the poles but found only in water included Sporichthyaceae, Spirosomaceae, Burkholderiaceae, and Caulobacteraceae; families found only in sediments were Intrasporangiaceae, Geobacteraceae, Gemmatimonadaceae, and Nitrosomonadaceae. Families found in both water and sediment included Microbacteriaceae, Flavobacteriaceae, Comamonadaceae, Rhodobacteraceae, and Sphingomonadaceae. In the case of Unknown Families, they were more prevalent in sediments at both poles than in water.

At the genus level, those exclusively found in the Arctic included *Fluviicola*, *Limnohabitans*, and *Sphingorhabdus* in water, while *Thiobacillus* was found only in Arctic sediments. In Antarctica, *Acinetobacter*, *Colwellia*, and *Pseudomonas* were exclusive to water, while *Winogradskyella*, *Granulosicoccus*, *Sphingomonas*, *Sulfitobacter*, and *Yoonia-Loktanella* were present in both water and sediments. Genera common to both poles, found only in water, included *hgcI clade*, *Ferruginibacter*, *Flavobacterium*, *Pseudarcicella*, *Polaromonas*, and *Polynucleobacter*. *Oryzihumus* was the only genus found in common between the two poles in sediments. Finally, unknown genera were found in higher proportions in sediments compared to water.

The nMDS plot constructed with ASVs data shows that the ASVs from water and sediment across all lakes are distinct from each other, except in the case of the Antarctic brackish lakes — Telefon (LT), Ballaneros (LB), and Extrematura (LE) — where, as observed in the plot, water and sediment are grouped together. Finally, the communities in the water of the Arctic lakes and the Antarctic lake Zapatilla (LZ) differ from those in other Antarctic lakes. Lastly, the communities in the Antarctic lakes Argentina (LA), Crater (LC), and Sofia (LS) were distinct from all others (Fig. 12).

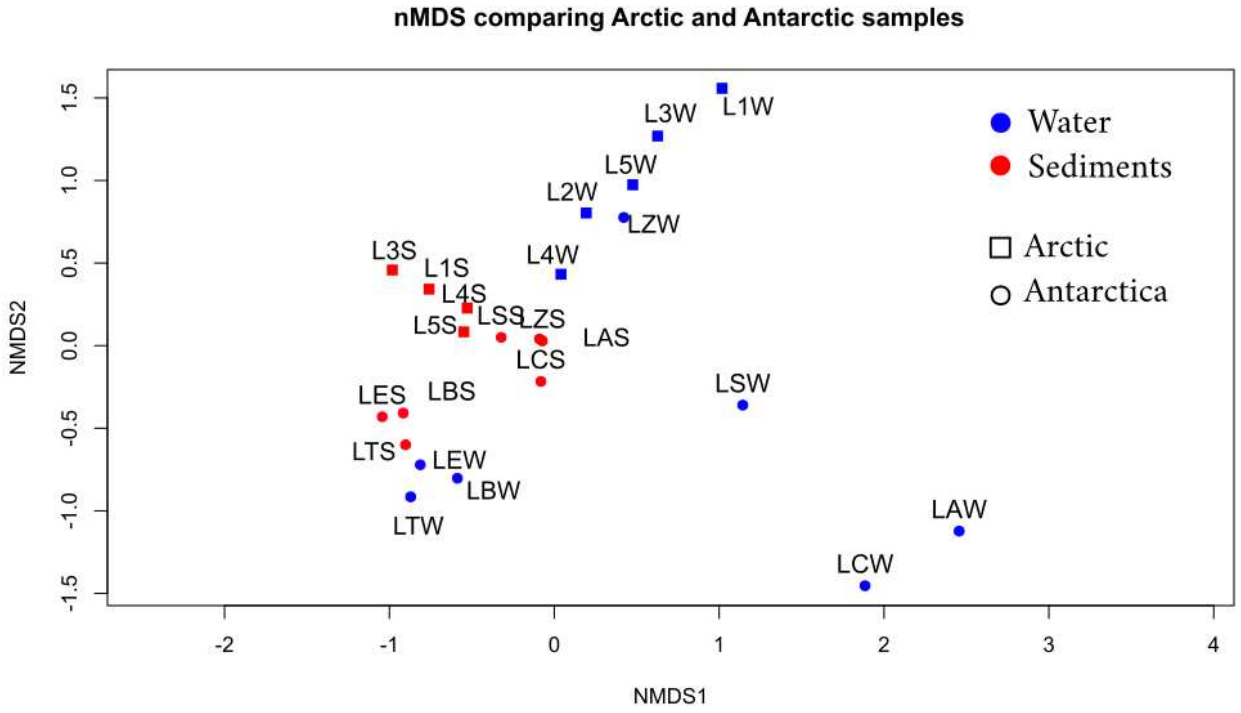


Fig. 12: nMDS plot constructed with ASVs detected in sediment and water of Arctic and Antarctic lakes.

4.1.4 Discussion

Based on obtained results, the data on prokaryotic community composition revealed important insights into Arctic and Antarctic lakes. While there were notable similarities, especially at phylum level, across the poles, significant differences were observed between water and sediment, also in the same lake. Within each region, some lakes, particularly the brackish ones in Antarctica, Telefon (LT), Ballaneros (LB), and Extremadura (LE), displayed similar microbial communities in both water and sediment. Additionally, the analysis highlighted that many taxa were shared between the lake matrices of the two poles, further underscoring these ecosystems' interconnectedness despite their geographical separation.

Starting from the phylum level, general results showed that the main retrieved groups were Actinobacteriota, Bacteroidota, Gammaproteobacteria, and Alphaproteobacteria. These findings aligned with previous studies documenting that bacterial communities in the water column and the sediment of polar regions was dominated by the same phyla retrieved in our analysis (Vick-Majors

et al., 2014; Wang et al., 2016; Gugliandolo et al, 2016; Li & Morgan-Kiss, 2019; Picazo et al., 2019). In particular, such of these phyla are known for their specific role in the ecosystem. Actinobacteriota plays a crucial role as biogeochemical cycles and bioremediation (Alvarez et al., 2017). Bacteroidota were largely known for their ability to degrade dissolved organic matter (Díez-Vives et al., 2019). Alphaproteobacteria were capable of degrading complex organic compounds in oligotrophic environment (Newton et al., 2011). Gammaproteobacteria are widely considered transient members deriving from anthropogenic or zoonotic sources (Newton et al., 2011). Between the two poles differences at phylum level were detected more in the sediments than in the water, For example, Desulfobacterota. This phylum plays a crucial role in anoxic sediment biogeochemical sulfur and iron cycling conditions (Greene, 2014; Lovley et al., 1993; Simon & Kroneck, 2013).

Most of the families identified were common between the two poles. The main families included Flavobacteriaceae, responsible for the degradation of complex organic matter (Barbeyron et al., 2016; Bennke et al., 2016); Sporichthyaceae, generally associated with the degradation of complex organic materials (Kim et al., 2006; Flint & Duncan, 2014; Coenye, 2014;); Comamonadaceae, known as denitrifying bacteria (Zang et al., 2020); Sphingomonadaceae, which played an important role in nutrient cycling, especially in oligotrophic environments (Aylward et al., 2013); and Rhodobacteraceae, involved in sulfur and carbon biogeochemical cycling (Pujalte et al., 2014). In contrast, few families exhibited region-specific distributions. Bacteroidetes vadinHA17, associated with anaerobic digestion and organic matter degradation (Jiang et al., 2011), was primarily found in Arctic samples, particularly within the sediments of Solvannet (L1) and Knudsenheia Lakes (L3), and to a lesser extent in the sediment of Tvillingvatnet Lake (L5) and both the water and sediment of Storvatnet Lake (L4). Conversely, Pseudomonadaceae, known for its role in nutrient recycling (Yonezuka et al., 2017), was detected in the water of Antarctic lakes, specifically Sofia (LS), Argentina (LA), and Crater Lakes (LC). Significant differences at the family level emerged when lakes with similar characteristics were compared between the poles. This was particularly true for the brackish Lakes, Knudsenheia (L3) (Arctic) compared with the Antarctic Extremadura (LE), Telefon

(LT), and Ballaneros Lakes (LB). In the Arctic Knudsenheia Lake (L3), the dominant families were Bacteroidetes vadinHA17, Sporichthyaceae, Crocinitomicaceae, Spirosomaceae, and Comamonadaceae. Conversely, the Antarctic brackish lakes were dominated by Rhodobacteraceae and Flavobacteriaceae. These findings are probably related to nutrient and oxygen availability which are key drivers of microbial community composition and distribution, shaping the observed differences between Arctic and Antarctic lakes. Variations in these factors create distinct niches that select specialized microbial groups adapted to specific conditions. For example, anaerobic environments may favor taxa like Bacteroidetes vadinHA17 (Mei et al., 2020).

A similar trend was observed when comparing the Arctic human-impacted lakes (Solvannet, L1, and Storvatnet, L4) with the Antarctic human-impacted Lakes (Argentina, LA, and Crater, LC). In the Arctic lakes, the dominant families were Comamonadaceae (15% and 10% respectively), Burkholderiaceae (9% and 5%), Bacteroidetes vadinHA17 (7% and 3%), Flavobacteriaceae (27% in Solvannet only), and Intrasporangiaceae (7% in Storvatnet only). The Antarctic human-impacted lakes were characterized by Pseudomonadaceae (40% and 6%), Sphingomonadaceae (6% and 23%), Caulobacteraceae (11% in Crater only), Moraxellaceae (11% in Crater only), and Intrasporangiaceae (7% in Argentina only). These findings are probably related to the specific types and levels of impact experienced by each lake. The abundance of Comamonadaceae or Burkholderiaceae was mainly related to higher nutrient loading, for example, coming from migrant animals frequenting the lakes (Ji et al., 2018). Conversely, another human-impacted lake with different stressors, such as anthropogenic or altered oxygen levels, might be selected for families like Sphingomonadaceae, which may be better adapted to those specific conditions (Newton et al., 2011). Most differences were retrieved at the genus level, and distinct communities were observed in Arctic and Antarctic lakes, particularly between water and sediments. In fact, in water, most retrieved genera were *hgcI clade* play a role in a carbon fixation (Ghylin et al., 2014), *Flavobacterium* know for degrading complex organic carbon (Bernardet & Bowman, 2006) and *Polynucleobacter* know for the degradation of dissolved organic matter (Watanabe et al., 2009), whereas in sediments retrieved genera such as

Oryzihumus play a role in the soil organic carbon mineralization (Fu et al., 2022). *Limnohabitans* is able to degrade organic carbon from algal exudates (Hahn et al., 2010) were found only in Arctic water samples, the abundance of these bacteria is often correlate to low-molecular-weight compounds in dissolved organic carbon (Šimek et al., 2010), maybe due to the influence of tundra vegetation and the small dimension of lakes and ponds where the dissolved organic matter is more abundant (Lim et al. 2001).

Instead, *Pseudomonas* members are important bacteria in the recycling of nutrients (Yonezuka et al., 2017), *Sphingomonas* affiliates have the capacity to degrade recalcitrant carbon sources (White et al., 1996), and *Sulfitobacter* members are heterotrophic marine species able to oxidize sulfite (Sorokin, 1995). They were all well represented in the Antarctic samples. In literature *Sulfitobacter* members require NaCl for their growth (Sorokin, 1995). Maybe it is for this reason that it was retrieved only in the brackish lakes of Antarctica. Contrary to our data, *Pseudomonas* and *Sphingomonas* have also been isolated in Arctic lakes (Zakharova et al., 2022).

In the brackish Arctic Lake Knudsenheia (L3), *hgcI clade*, *Flavobacterium*, *Fluviicola*, *Pseudarcicella*, and *Limnohabitans* were the dominant genera. The Antarctic brackish lakes Telefon (LT), Ballaneros (LB), and Extremadura (LE), were characterized by *Yoonia-Loktanella*, *Sulfitobacter*, *Polaribacter*, *Maribacter*, and *Granulosicoccus*. *Flavobacterium* and *Polynucleobacter* dominated in human-impacted Arctic lakes, while *Pseudomonas* and *Sphingomonas* were prevalent in the Antarctic lakes. These differences likely reflect variations in environmental conditions and geographical isolation. Further research is needed to understand the specific ecological roles of these genera and the factors driving their distributions (Rochera & Camacho, 2019).

It should also be noted that even at the genus level, there were few genera of notable proportion. This result was likely influenced by the high percentage of unknown genera (average 26 and 21% for Arctic and Antarctic, respectively), especially in sediment samples.

From the results of Lui et al. (2021), who compared bacterial communities from Arctic and Antarctic lakes, it was noted that only 2.5% of the communities were shared between the two areas. Similarly, the study by Daniel et al., (2016) highlighted that bacterial communities in Antarctic and Arctic lakes revealed how geographical distance and local limnological profiles (e.g., temperature, conductivity, pH, and nutrient levels) played an important role in shaping the spatial distributions of bacterial communities (Logares et al., 2013; Comte et al., 2018; Yang et al., 2019). Conversely, in our findings, microbial communities showed more similarities between Arctic and Antarctic samples, but more relevant distinctions were found among water and sediment samples. This could be dependent on Arctic and Antarctic lakes exhibiting distinct influence by variations in environmental conditions, geographical isolation, and localized impacts (Liu et al., 2021).

4.2 Isolation and characterization of heavy metal tolerant bacterial strains

4.2.1 Arctic lakes

4.2.1.1 Bacterial isolation

A total of 58 bacterial strains were isolated: 50 from the Fe-enrichments (i.e., 13 from Lake Solvannet, L1, 11 from Lake Glacier, L2, 16 from Knudsenheia Lake, L3, and 10 from Storvatnet Lake, L4, respectively), and 8 from the Hg-enrichments, all from Lake Storvatnet (L4).

4.2.1.2 Bacterial tolerance at increasing concentrations of heavy metals

Isolates (n= 50) from Fe-enrichments were tested at higher Fe concentrations, i.e., 2500 ppm and 5000 ppm than in the isolation medium. Overall, 15 isolates was able to grow at least one of the Fe concentrations evaluated. They were mostly affiliated with the phylum Gammaproteobacteria, all belonging to the genus *Pseudomonas*. The identified species included *P. antartica* and *P.*

extremaustralis. Additionally, two strains were affiliated with the phylum Betaproteobacteria, specifically within the genus *Janthinobacterium*, while one strain belonged to the phylum Bacillota and was identified as *Carnobacterium maltaromaticum*. Unfortunately, the identification was not successful for four strains (i.e., S2A-1, S2A-4, S2A-5 and S4A-2). Nucleotide sequences have been deposited in the NCBI GenBank database (see Tab. 3)

Tab. 3: Identification of Fe-tolerant bacterial isolates by the 16S rRNA gene sequencing.

	Sample ID	Affilated organisms	Similarity (%)	accession number	Phylum	Order	Family	Genus	Species
Solvannet Lake (L1)	S1A-14	*Pseudomonas antarctica*	100.00	PQ687016	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*antarctica*
Glacier Lake (L2)	S2-1	*Not identified*							
	S2A-2	*Carnobacterium maltaromaticum*	100.00	PQ686999	Bacillota	Lactobacillales	Carnobacteriaceae	*Carnobacterium*	*maltaromaticum*
	S2A-4	*Not identified*							
	S2A-5	*Not identified*							
	S2A-6	*Janthinobacterium sp.*	99.90	PQ687000	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	
	S2A-7	*Pseudomonas extremaustralis*	100.00	PQ687001	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*extremaustralis*
	S2A-8	*Pseudomonas extremaustralis*	99.80	PQ687002	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*extremaustralis*
Knudsenheia Lake (L3)	S3A-2	*Pseudomonas* sp	99.81	PQ687003	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	
	S3A-11	*Pseudomonas* sp	99.73	PQ687004	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	
Storvatnet Lake (L4)	S4A-1	*Pseudomonas* sp	99.54	PQ687008	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	
	S4A-2	*Not identified*							
	S4A-4	*Pseudomonas antarctica*	99.62	PQ687005	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*antarctica*
	S4A-7	*Janthinobacterium* sp.	88.93	PQ687006	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	
	S4A-10	*Pseudomonas antarctica*	97.91	PQ687007	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*antarctica*

For example, *Carnobacterium* sp. S2A-2 better tolerated 2500 ppm of Fe than 5000 ppm, wherese strain S1A-14 (*Pseudomonas* sp.) exhibited grew up to 5000 ppm of the metal. The strains that showed the highest tolerance to both concentrations were *Pseudomonas* spp. S2A-1, S2A8, and S4A-10 (Table X, Lista ceppi). Among the positive strains, one originated from Solvannet Lake (L1), seven from Glacier Lake (L2), two from Knudsenheia Lake (L3), and five from Storvatnet Lake (L4). In contrast, for the mercury tests, none of the strains tested showed tolerance at the tested concentrations (data not shown). Isolates (n= 8) from Hg-enrichments affiliated to *Pseudomonas mandeli* were tested at concentrations of 250 and 500 ppm of the metal. None of the strains was able to grow at the tested concentrations (*data not shown*).

Tab 4: Bacterial isolates growing at 2500 and/or 5000 ppm of Fe. (+) =from 0.05 to 0.1, + => 0.1, ++=> 0.2.

	ID strain	**Fe 2500 ppm**	**Fe 5000 ppm**	Affilated organisms
Solvannet Lake (L1)	S1A-14	+	+	*Pseudomonas antarctica*
Glacier Lake(L2)	S2A-1	+	+	Not identified
	S2A-2	+	+	*Carnobacterium maltaromaticum*
	S2A-4	+	(+)	Not identified
	S2A-5	+	(+)	Not identified
	S2A-6	(+)	(+)	*Janthinobacterium* sp.
	S2A-7	+	+	*Pseudomonas extremaustralis*
	S2A-8	++	+	*Pseudomonas extremaustralis*
Knudsenheia Lake (L3)	S3A-2	+	+	*Pseudomonas* sp
	S3A-11	+	+	*Pseudomonas* sp
Storvatnet Lake (L4)	S4A-1	(+)	(+)	*Pseudomonas* sp
	S4A-2	(+)	+	Not identified
	S4A-4	+	+	*Pseudomonas antarctica*
	S4A-7	+	(+)	*Janthinobacterium* sp.
	S4A-10	+	+	*Pseudomonas antarctica*

4.2.1.3 Screening for multitolerance toward heavy metals

The strains that resulted positive in the previous experiments were further analyzed. Two strains, specifically *Pseudomonas* spp. S2A-8 and S3A-11, exhibited tolerance to both Cu and Hg, growing at 1000 ppm of Cu and 250 ppm of Hg. Additionally, several strains displayed a higher tolerance to Hg. For instance, *Pseudomonas* sp. S1A-14 and strain S2A-5 (not identified) tolerated Hg at 100, 250 and 500 ppm. Strains *Pseudomonas* spp. S2A-7, S3A-2, S4A-1, S4A-2 (not identified), S4A-4 (not identified), S4A-7 (not identified), and S4A-10 (not identified) demonstrated tolerance to Hg at 250 ppm (see Tab. 5). Conversely, strains S2A-1 (not identified), S2A-2 (not identified), *Carnobacterium* sp. S2A-4, and *Janthinobacterium* sp. S2A-6 showed no growth on the tested metals.

Tab. 5: Screening of selected isolates for multitolerance. (+), weak response.

	ID strain	**Cu 1000 ppm**	**Cu 2500 ppm**	**Cu 5000 ppm**	**Hg 100 ppm**	**Hg 250 ppm**	**Hg 500 ppm**	**Affilated organisms**
Solvannet Lake (L1)	S1A-14	-	-	-	(+)	(+)	(+)	*Pseudomonas antarctica*
Glacier Lake (L2)	S2A-1	-	-	-	-	-	-	Not identified
	S2A-2	-	-	-	-	-	-	*Carnobacterium maltaromaticum*
	S2A-4	-	-	-	-	-	-	Not identified
	S2A-5	-	-	-	(+)	(+)	(+)	Not idetified
	S2A-6	-	-	-	-	-	-	*Janthinobacterium* sp.
	S2A-7	-	-	-	(+)	+	-	*Pseudomonas extremaustralis*
	S2A-8	(+)	-	-	(+)	(+)	-	*Pseudomonas extremaustralis*
Knudsenheia Lake (L3)	S3A-2	-	-	-	(+)	(+)	-	*Pseudomonas* sp
	S3A-11	(+)	-	-	+	+	-	*Pseudomonas* sp
Storvatnet Lake (L4)	S4A-1	-	-	-	+	+	-	*Pseudomonas* sp
	S4A-2	-	-	-	+	+	-	Not identified
	S4A-4	-	-	-	+	+	-	*Pseudomonas antarctica*
	S4A-7	-	-	-	+	+	-	*Janthinobacterium* sp.
	S4A-10	-	-	-	+	+	-	*Pseudomonas antarctica*

4.2.1.4 Mercury and iron sequestration rate

Six of the most promising strains from previous tests were evaluated for their sequestration rates towards Hg and Fe. As it is shown in Fig. 13.a, the strains did not exhibit significant Hg sequestration abilities. Among the best-performing strains, S2A-5 (not identified) sequestered approximately the 15% of the metal, followed by *Pseudomonas* sp. S1A-14 (12%) and S2A-8 (not identified; 10%). All the remaining isolates showed a sequestration rate below 5%.

Similarly, analyzed strains showed a limited ability to sequester Fe. The best-performing strain was *Pseudomonas* sp. S2A-8, which achieved a sequestration rate of 12%. The other strains showed a sequestration rates below 5% (see Fig. 13.b).

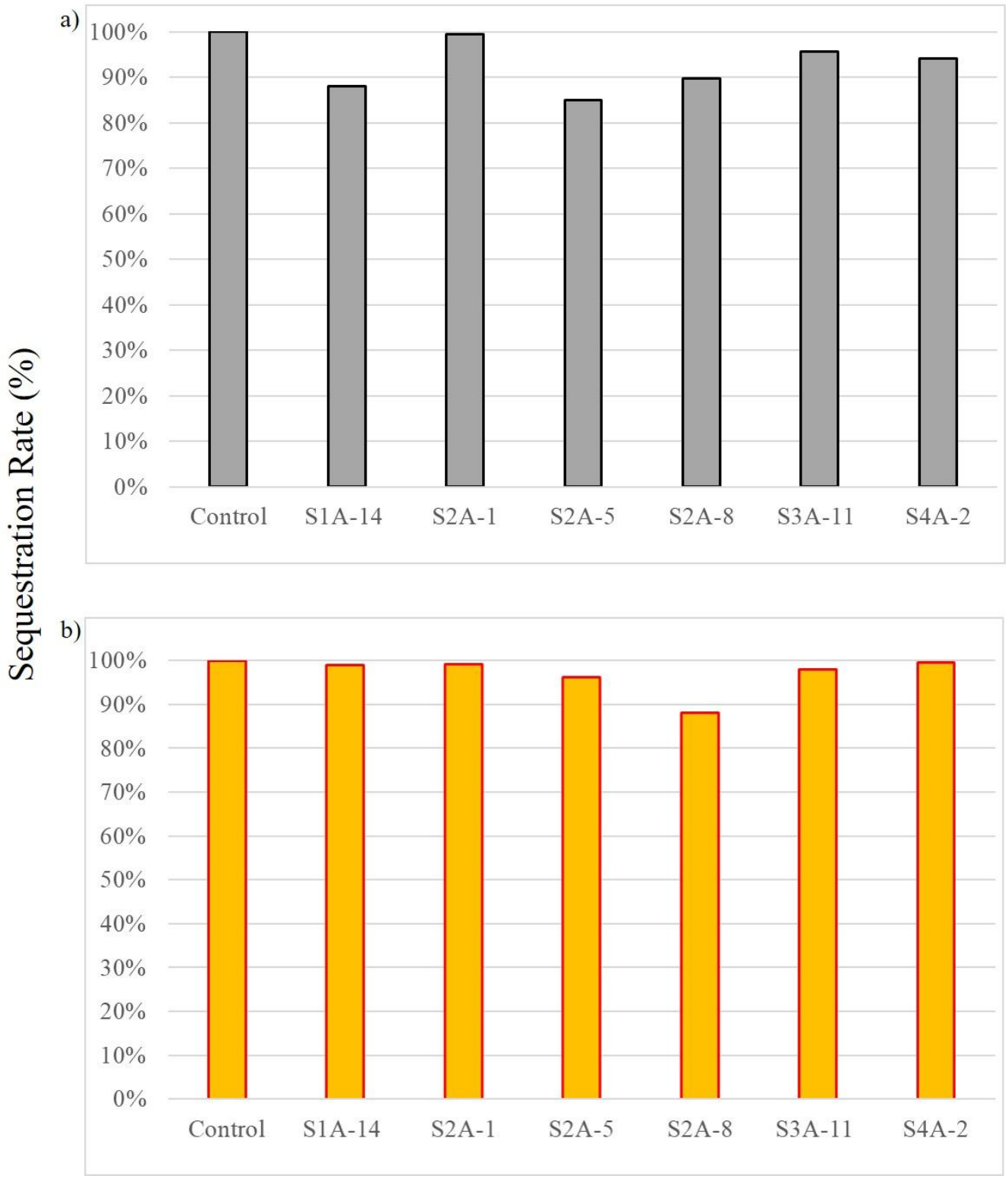


Fig 13: a) Hg Sequestration Rate Percentage b) Fe Sequestration Rate Percentage

4.2.2 Antarctic lakes

4.2.2.1 Bacterial isolation

Due to the absence of growth, strain isolation was performed from the plates corresponding to the negative control, i.e., the medium without added metal. A total of 43 strains were isolated: specifically, 40 from Fe enrichment, including 3 from Argentina Lake, 7 from Zapatilla Lake, 4 from Extremadura Lake, and 13 each from Telefon Lake and Ballaneros Lake. Additionally, 3 strains were isolated from Cu enrichments, all originating from Lake Ballaneros.

4.2.2.2 Bacterial tolerance at increasing concentrations of heavy metals

Isolates from Fe-enrichments were tested of 2500 ppm and 5000 ppm of Fe. Among them, 10 strains showed positive results. They were mainly affiliated to the phylum Actinobacteriota. Specifically, two strains (i.e., AZA-8 and AZA-9) were closely related to *Subtercola frigoramans*. Two strains (i.e., ATA-13 and ABA-13) were affiliated with the genus *Arthrobacter* (*A. alpinus* and *A. livingstonensis*, respectively). Additionally, two strains (i.e., AAA-2 and AAA-4) were closely related to *Pseudoarthrobacter scleromae*. Finally, two strains (i.e., AZA-2 and AZA-4) were affiliated with the phylum Betaproteobacteria, both belonging to the genus *Janthinobacterium*. The affiliation could not be determined for two strains (ABA-4 and AZA-3). Nucleotide sequences have been deposited in the NCBI GenBank database (see Tab. 6). The strains with the highest Fe-tolerance at both concentrations were *Pseudarthrobacter* sp. AAA-2, *Janthinobacterium* spp. AZA-2, and AZA-4. Meanwhile, AA2, AAA-4, AZA-2, AZA-3, AZA-4, AZA-8, and AZA-9 demonstrated greater tolerance at the 2500 ppm concentration, while the remaining 3 strains showed lower tolerance at both concentrations (Tab. 7).

Tab 6: Identification of Fe-tolerant bacterial isolates by the 16S rRNA gene sequencing.

	Sample ID	Affilated organisms	Affiliation %	accession number	Phylum	Order	Family	Genus	Species
Zapatilla Lake (LZ)	AZA-2	*Janthinobacterium svalbardensis*	99.90	PQ687013	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	*svalbardensis*
	AZA-3	Not identified							
	AZA-4	*Janthinobacterium sp.*	99.52	PQ687014	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	
	AZA-8	*Subtercola frigoramans*	97.62	PQ687015	Actinomycetes	Micrococcales	Microbacteriaceae	*Subtercola*	*frigoramans*
	AZA-9	*Subtercola frigoramans*	99.52	PQ687016	Actinomycetes	Micrococcales	Microbacteriaceae	*Subtercola*	*frigoramans*
Telefon Lake (LT)	ATA-13	*Arthrobacter alpinus*	99.12	PQ687012	Actinomycetes	Micrococcales	Micrococcaceae	*Arthrobacter*	*alpinus*
Ballaneros Lake (LB)	ABA-4	Not identified							
	ABA-13	*Arthrobacter livingstonensis*	99.55	PQ687011	Actinomycetes	Micrococcales	Micrococcaceae	*Arthrobacter*	*livingstonensis*
Argentina Lake (LA)	AAA-2	*Pseudarthrobacter scleromae*	99.90	PQ687009	Actinomycetes	Micrococcales	Micrococcaceae	*Pseudarthrobacter*	*scleromae*
	AAA-4	*Pseudarthrobacter scleromae*	99.82	PQ687010	Actinomycetes	Micrococcales	Micrococcaceae	*Pseudarthrobacter*	*scleromae*

Tab 7: Bacterial isolates growing at 2500 and/or 5000 ppm of Fe. (+) =from 0.05 to 0.1, + => 0.1, ++=> 0.2.

	ID strain	Fe 2500 ppm	Fe 5000 ppm	Affilated organisms
Ballaneros Lake (LB)	ABA-4	(+)	(+)	Not identified
	ABA-13	(+)	(+)	*Arthrobacter livingstonensis*
Argentina Lake (LA)	AAA-2	++	+	*Pseudarthrobacter scleromae*
	AAA-4	+	(+)	*Pseudarthrobacter scleromae*
Zappatilla Lake (LZ)	AZA-2	++	+	*Janthinobacterium svalbardensis*
	AZA-3	++	(+)	Not identified
	AZA-4	++	+	*Janthinobacterium* sp.
	AZA-8	++	(+)	*Subtercola frigoramans*
	AZA-9	+	(+)	*Subtercola frigoramans*
Telefon Lake (LT)	ATA-13	(+)	(+)	*Arthrobacter alpinus*

4.2.2.3 Screening for multitolerance toward heavy metals

The multitolerance test showed that Hg was generally the most tolerated metal among the tested strains. While none of the strain showed an optimal growth, some of them demonstrated a notable tolerance. Strain ABA-4 (not identified) grew at all tested Cu concentrations and at 100 ppm and 250 ppm of Hg. Similarly, AZA-8 (*Subtercola frigoramans*) grew at all tested Hg concentrations and at 1000 ppm and 2500 ppm of Cu. Additionally, strains ABA-13 (*Arthrobacter livingstonensis*) and AAA-2 (*Pseudarthrobacter scleromae*) demonstrated a evident tolerance to Hg at 100 and 250 ppm. The strains AAA-4 (*Pseudarthrobacter scleromae*), AZA-2 (*Janthinobacterium svalbardensis*), AZA-3 (not identified) and AZA-4 (*Janthinobacterium* sp.) also showed a tolerance to Hg at the concentration of 100 ppm and. Conversely, strains AZA-9 (*Subtercola frigoramans)* and ATA-13 (*Arthrobacter alpinus*) showed no tolerance to any of the tested metals (Tab. 8).

Tab 8: Table of results of multitolerance of heavy metal, (+), weak response.

	ID strain	Cu 1000 ppm	Cu 2500 ppm	Cu 5000 ppm	Hg 100 ppm	Hg 250 ppm	Hg 500 ppm	Affilated organisms
Ballaneros Lake (LB)	ABA-4	(+)	(+)	(+)	(+)	(+)	-	Not identified
	ABA-13	-	-	-	+	+	-	*Arthrobacter livingstonensis*
Argentina Lake (LA)	AAA-2	-	-	-	+	+	-	*Pseudarthrobacter scleromae*
	AAA-4	-	-	-	+	(+)	-	*Pseudarthrobacter scleromae*
Zapatilla Lake (LZ)	AZA-2	-	-	-	+	(+)	-	*Janthinobacterium svalbardensis*
	AZA-3	-	-	-	+	(+)	-	Not identified
	AZA-4	-	-	-	(+)	(+)	-	*Janthinobacterium* sp.
	AZA-8	(+)	(+)	-	(+)	(+)	(+)	*Subtercola frigoramans*
	AZA-9	-	-	-	-	-	-	*Subtercola frigoramans*
Telefon Lake (LT)	ATA-13	-	-	-	-	-	-	*Arthrobacter alpinus*

4.2.2.4 Mercury and iron sequestration rate

Finally, based on the results of the previous tests, three strains were selected to assess their Hg and Fe sequestration rates. For Hg, no strain demonstrated excellent abilities to sequester this metal; the best performing strain was *Pseudarthrobacter* sp. AAA-2, which sequestered 5% of the mercury (Fig. 14a). Similarly, no exceptional results were obtained for Fe sequestration. The best performer, strain *Arthrobacter* sp. ABA-13, achieved a sequestration rate of 14%, while the other two strains showed almost negligible metal sequestration rates (Fig. 14b).

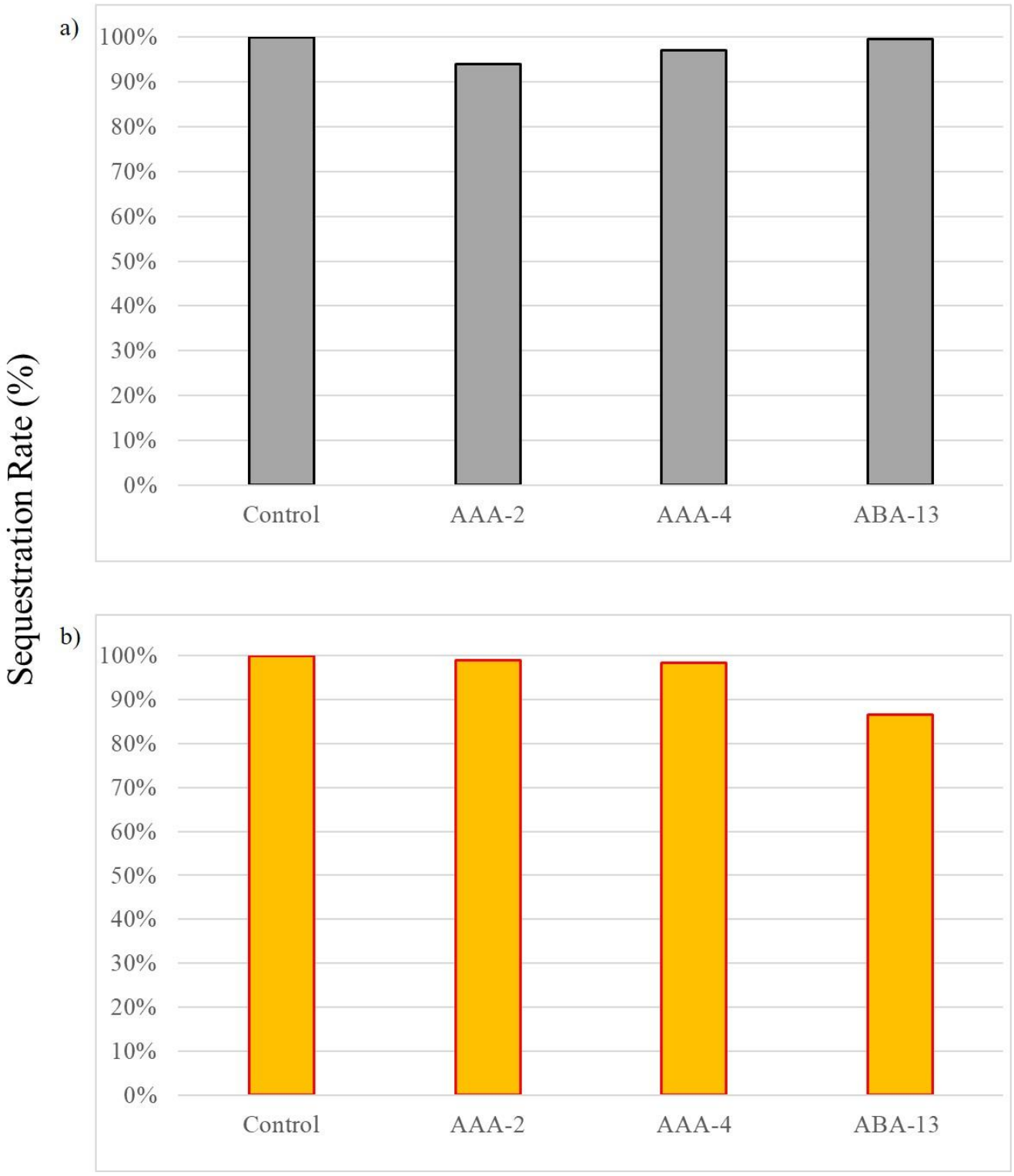


Fig .14: a) Hg Sequestration Rate Percentage b) Fe Sequestration Rate Percentage

4.2.3 Phylogenetic analysis of heavy metal tolerant strains

Overall,the phylogenetic tree (Fig. 15) revealed two major groups: one clustering strains from Arctic lakes, and another comprising strains from Antarctic lakes, with some exceptions. In particular, the first main group was divided into three subgroups; the first including the strains that were close to genus *Pseudomonas,* all from Arctic lakes; the second one composed of the strains that were close to the genus *Janthinobacterium*, two strains from Arctic lakes (S2A-6, S4A-7) and two strains from Antarctic lakes (AZA-2, AZA-4). Finaly the third group, distant to the other two groups was close to the genus *Carnobacterium* with one strain from Arctic lake (S2A-2).

Regarding the second main group clustered into two subgroups, one belonging to strains close to *Subtercola* genus, including two strains from Antarctic isolates (AZA-8 and AZA-9), and the second subgroup close to the genus *Arthrobacter* including four Antarctic strains (AAA-4, AAA-2, ATA-13 and ABA-13).

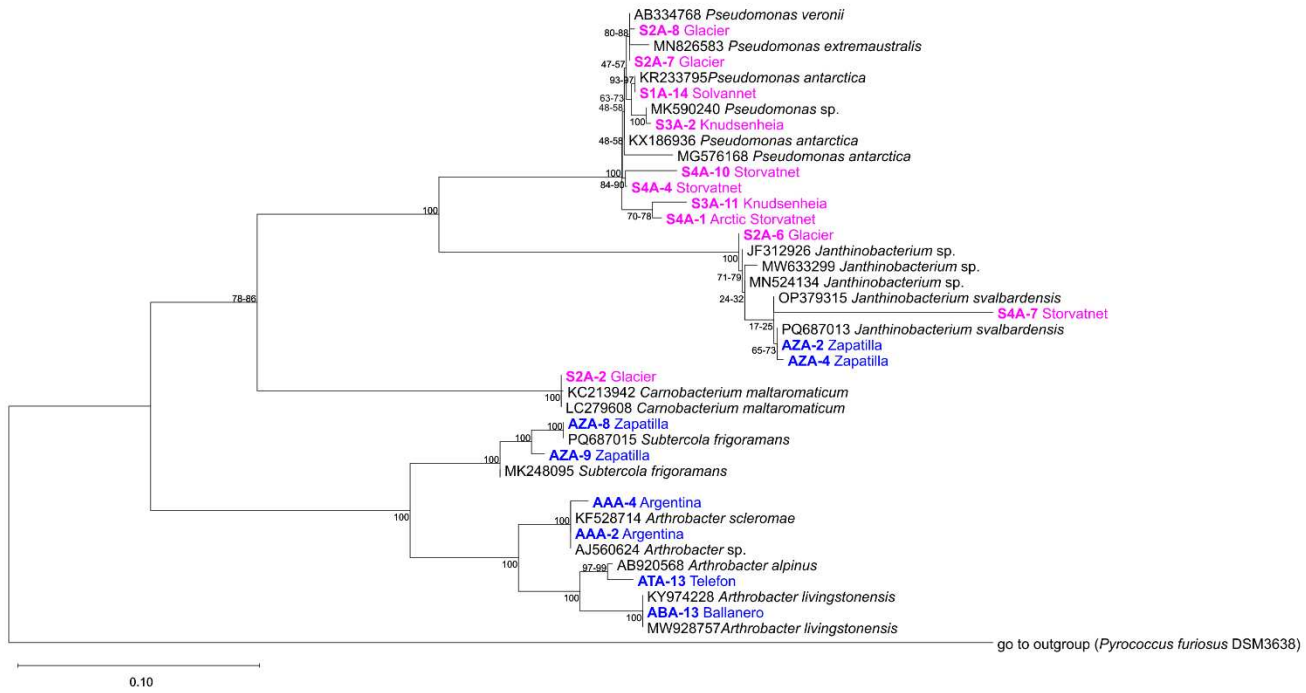


Fig. 15: Phylogenetic tree of Fe-tolerant bacterial isolates (in pink Arctic strains; in bleu Antarctic strains).

4.2.4 Discussion

Evaluating metal resistance in bacterial strains isolated from Arctic and Antarctic environments demonstrated interesting patterns. In this study, 58 Arctic bacterial and 43 Antarctic bacterial were isolates from enrichment additionated with to Cu-, Fe-, and Hg- at 4°C. Tests at higher concentrations of these metals showed that Fe was better tolerated than Cu and Hg. These findings are in line with previous studies reporting iron resistance as a common trait in environmental bacteria from cold regions, likely due to its essential role in microbial metabolism and availability in sediment environments (Ccorahua-Santo et al., 2017; Soto-Padilla et al., 2018). In the frame of the multi-tolerance test, mercury was generally the most tolerated metal, with strains such as ABA-4 and (*Subtercola frigoramans*) AZA-8 demonstrating growth at multiple concentrations. This finding reflects the potential adaptation of these bacteria to mercury exposure in their environment, possibly linked to the presence of mercury-binding proteins or efflux systems. Mercury resistance was more prevalent in Antarctic strains compared to Arctic strains, with several strains tolerating mercury at concentrations up to 250 ppm. This highlights the potential influence of regional environmental factors and the selective pressures of heavy metal presence in different ecosystems (Romaniuk et al., 2018). The identification of positive, promising strains showed that in the Arctic they were mainly represented by Gammaproteobacteria and Bacillota, mainly dominated by the genus *Pseudomonas*. In contrast, Antarctic strains were more diverse within Actinobacterota, with multiple genera such as *Subtercola*, *Arthrobacter*, and *Pseudarthrobacter* represented. Arctic and Antarctic samples included strains from the genus *Janthinobacterium*, with *J. svalbardensis* was detected in both environments (Kleinteich et al., 2017; Tytgat et al., 2014). These differences suggest that Arctic environments may select for taxa like *Pseudomonas*, known for their metabolic versatility; instead, Antarctic conditions seem to favor Actinobacterota, which are recognized for their ability to withstand extreme conditions, including nutrient limitation and low temperatures (Vyverman et al., 2010). The differences in taxonomic composition likely reflect the distinct environmental pressures and microbial niches in

these polar regions. The taxa belonged of *Pseudomonas* are known for their ability to reduce a wide variety of metals (Blake et al., 1993; Malla et al., 2018; Zahri et al., 2021; Kümmerli, 2023; Prieto-Fernández et al., 2024). The genus *Janthinobacterium* (among Betaproteobacteria) was common to both regions, whereas a single *Carnobacterium* representative (among Firmicutes) was isolated from an Arctic lake. *Pseudomonas* and *Janthinobacterium* were among prevalent genera harboring resistome genes (genes related to antibiotic, biocide, and heavy metal resistances) in a study carried out in marine sediments at Deception Island (Centurion et al., 2022). *Janthinobacterium* was reported to be tolerant to heavy metals, such as Ag, Cu, Hg, Pb and Ni (Shi et al. 2008). Instead, in another study the relative abundance of *Janthinobacterium* was inversely correlated to the highly pollution of samples (Yin et al. 2015). The tolerance of *Carnobacterium* members to heavy metals, such as Cd and Sb, have been demonstrated (Butrimienė et al., 2022), also in cold environments (Prieto-Fernández et al., 2024). Heavy metal tolerance in cold-adapted *Arthrobacter* members has been frequently documentated (e.g., Romaniuk et al., 2018; Abdulrasheed et al., 2020; Darham et al., 2021). A comprehensive genome sequencing on the psychrotolerant *Arthrobacter* sp. PAMC25284 from Antarctic revealed the presence of genes involved with copper resistance (Karmacharya et al., 2022). *Pseudoarthrobacter* members, from warmer areas, have seldom been reported as heavy metal tolerant (Park et al., 2021; Singh & Narzary, 2021). Finally, to the best of our knowledge we report for the first time the ability of the genus *Subtercola* to grow in the presence of heavy metals.

The assessment of metal sequestration revealed limited capacities among bacterial strains from Arctic and Antarctic environments. Antarctic strains demonstrated slightly better sequestration potential regarding iron, with *Arthrobacter livingstonensis* (ABA-13) achieving the highest rate at 14%. In contrast, the best-performing Arctic strain, *Pseudomonas extremaustralis* (S2A-8), reached a sequestration rate of 12%. Mercury sequestration was minimal across both regions, but in contrast with iron sequestration rate. In fact, Antarctic strain *Pseudarthrobacter scleromae* (AAA-2) achieved 5%, while Arctic strains like *Pseudomonas extremaustralis* (S2A-8) sequestered 10% of

mercury. These results suggest that while polar bacteria possess resistance to heavy metals, their sequestration efficiency remains modest, potentially limiting their direct application in bioremediation (De Souza et al., 2007; Neethu et al., 2015; Giovanella et al., 2017). The differences in metal sequestration abilities between Arctic and Antarctic strains may reflect their distinct taxonomic compositions and ecological adaptations (Romaniuk et al., 2018). Whereas, the predominance of Actinobacteriota among Antarctic strains may confer a slight advantage in iron sequestration due to their robust cell wall structures, which can facilitate metal binding (Romaniuk et al., 2018). In contrast, Arctic strains, dominated by *Pseudomonas*, may prioritize resistance mechanisms such as efflux systems over sequestration. Our results underscore the importance of cold-adapted bacterial strains in understanding metal resistance mechanisms in extreme environments. While their sequestration capabilities are modest, the genetic determinants of resistance in these taxa could provide valuable insights for developing biotechnological applications in heavy metal bioremediation. Future work should focus on characterizing resistance genes and their regulatory pathways and exploring co-culture strategies to enhance sequestration rates. Main results are part of a paper recently published in Microrganisms (doi: 10.3390/microorganisms13020389).

4.3 Isolation and characterization of polychlorinated biphenyl degrading bacterial strains

4.3.1 Arctic Lakes

4.3.1.1 Strain isolation

A total of 46 strains were isolated from the biphenyl enrichment, of which 16 strains came from Lake Solvannet, 14 from Glacier Lake, 7 from Lake Knudsenheia, and finally 9 from Lake Storvatnet.

4.3.1.2 Test for polychlorinated biphenyl oxidation

A total of 24 (out of 46) strains grew in the presence of aroclor biphenyl (approximately 52%). Among these, 18 strains showed the presence of the *bph*A gene and were identified by the 16S rRNA gene sequencing (Tab. 9). Most of these strains belonged to the phylum Gammaproteobacteria, all within the genus *Pseudomonas*. Two strains belonged to the species *P. baetica*, 3 were affiliated to *P. fluorescens*, another 3 to *P. gessardii*, an additional 3 to *P. jessenii*, 1 belonging to the species *P. libanensis*, 2 to *P. paraversuta*, and finally 1 to *P. pictorum*. In contrast, the only strain belonging to the phylum Betaproteobacteria was affiliated with the species *Janthinobacterium lividum*. Unfortunately, the affiliation could not be determined for two strains (S3D-1 and S3D-2).

Nucleotide sequences have been deposited in the NCBI GenBank database (see Tab. 8)

Tab. 8: Identification of PCB-oxidant bacterial isolates by the 16S rRNA gene sequencing.

	Sample ID	Affilated organisms	Affiliation %	Accession number	Phylum	Order	Family	Genus	Species
Solvannet Lake (L1)	S1D-1	*Pseudomonas pictorum*	99.91	PQ686980	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Stenotrophomonas*	*pictorum*
	S1D-8	*Pseudomonas paraversuta*	99.91	PQ686983	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*paraversuta*
	S1D-9	*Pseudomonas fluorescens*	99.81	PQ686986	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*fluorescens*
	S1D-11	*Pseudomonas paraversuta*	100	PQ686990	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*paraversuta*
Glacier Lake (L2)	S2D-5	*Pseudomonas gessardii*	100	PQ686993	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*gessardii*
	S2D-6	*Pseudomonas libanensis*	100	PQ686996	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*libanensis*
	S2D-8	*Janthinobacterium lividum*	99.67	PQ686975	Betaproteobacteria	Burkholderiales	Oxalobacteraceae	*Janthinobacterium*	*lividum*
	S2D-10	*Pseudomonas baetica*	98.82	PQ686978	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*baetica*
	S2D-11	*Pseudomonas jessenii*	100	PQ686981	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*jessenii*
	S2D-14	*Pseudomonas gessardii*	99.91	PQ686984	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*gessardii*
Knudsenheia Lake (L3)	S3D-1	*Not identified*							
	S3D-2	*Not identified*							
	S3D-4	*Pseudomonas gessardii*	99.91	PQ686987	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*gessardii*
Lake Storvatnet (L4)	S4D-3	*Pseudomonas jessenii*	100	PQ686991	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*jessenii*
	S4D-5	*Pseudomonas jessenii*	100	PQ686994	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*jessenii*
	S4D-6	*Pseudomonas fluorescens*	99.91	PQ686997	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*fluorescens*
	S4D-7	*Pseudomonas fluorescens*	100	PQ686976	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*fluorescens*
	S4D-8	*Pseudomonas baetica*	99.91	PQ686979	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*baetica*

Tab. 9: Growth in the presence of Aroclor 1242 and presence of *bph*A gene, (+), weak response

	ID strain	Grotwh on aroclor 1242	*bph*A	Affilated organisms
Solvannet Lake (L1)	S1D-1	(+)	+	*Pseudomonas pictorum*
	S1D-3	(+)	-	*Not identified*
	S1D-5	(+)	-	*Not identified*
	S1D-7	(+)	-	*Not identified*
	S1D-8	+	+	*Pseudomonas paraversuta*
	S1D-9	+	+	*Pseudomonas fluorescens*
	S1D-11	(+)	+	*Pseudomonas paraversuta*
Glacier Lake (L2)	S2D-5	(+)	+	*Pseudomonas gessardii*
	S2D-6	(+)	+	*Pseudomonas libanensis*
	S2D-8	(+)	+	*Janthinobacterium lividum*
	S2D-10	(+)	+	*Pseudomonas baetica*
	S2D-11	+	+	*Pseudomonas jessenii*
	S2D-14	+	+	*Pseudomonas gessardii*
Knudsenheia Lake (L3)	S3D-1	+	+	*Not identified*
	S3D-2	+	+	*Not identified*
	S3D-3	(+)	-	*Not identified*
	S3D-4	+	+	*Pseudomonas gessardii*
	S3D-5	+	-	*Not identified*
	S3D-12	+	-	*Not identified*
Lake Storvatnet (L4)	S4D-3	+	+	*Pseudomonas jessenii*
	S4D-5	(+)	+	*Pseudomonas jessenii*
	S4D-6	(+)	+	*Pseudomonas fluorescens*
	S4D-7	(+)	+	*Pseudomonas fluorescens*
	S4D-8	(+)	+	*Pseudomonas baetica*

4.3.1.3 PCB sequestration rate

Based on the tests described above, 7 bacterial strains were selected to assess their PCB (Aroclor 1242) degradation capability. Two strains, namely S3D-2 (not identified) and *Pseudomonas* sp. S3D-5, from Knudsenheia Lake were particularly effective (Fig. 16).

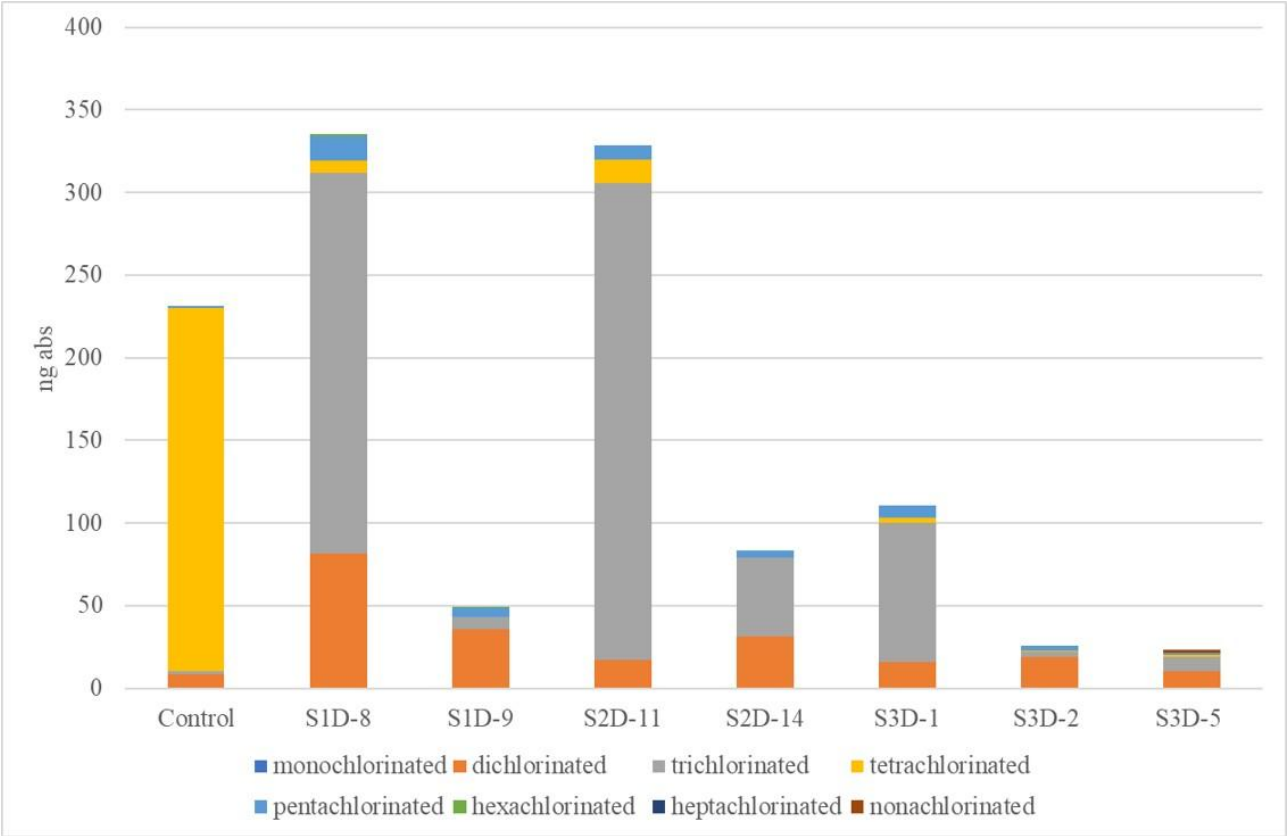


Fig. 16: PCB degradation rate of Arctic bacterial strains.

When examining specific PCB groups, a slight increase in monochlorinated PCBs, particularly PCB-1, was observed in comparison to the control (Fig. 17a). PCB-11 levels increased in all tested strains except for S3D-5 (not identified), which maintained a quantity like the control (Fig. 17b). For trichlorinated PCBs, the formation of PCB-16, absent in the control, was detected in (*Pseudomonas paraversuta*) S1D-8, (*Pseudomonas jessenii*) S2D-11, and (not identified) S3D-1 strains (Fig. 17c). However, strain (*Pseudomonas gessardii*) S2D-14 showed no traces of PCB-16 but was unique in forming PCB-18. Tetrachlorinated PCBs showed the most significant degradation, with complete removal in strains (*Pseudomonas fluorescens*) S1D-9, (*Pseudomonas gessardii*) S2D-14, and (not identified) S3D-2 (Fig. 17d). The other strains evidenced only small residual amounts compared to the control. In contrast, pentachlorinated PCBs increased in all samples except (not identify) S3D-5, where levels remained unchanged from the control, despite partial transformation (Fig. 17e). However, the overall levels of pentachlorinated PCBs across all strains were minimal relative to the

total. Finaly, small amounts of hexachlorinated PCBs were detected in strains (*Pseudomonas paraversuta*) S1D-8 and (Pseudomonas fluorescens) S1D-9 (Fig. 17f), while nonachlorinated PCBs were detected in (not identified) S3D-5 (Fig. 17g). Overall, all the tested strains showed great efficiency in degrading tetrachlorinated PCBs, though some strains generated byproducts that contributed to an increase in PCB concentration in the solution. Strain (not identify) S3D-2 emerged as the most effective when considering degradation across all PCB categories.

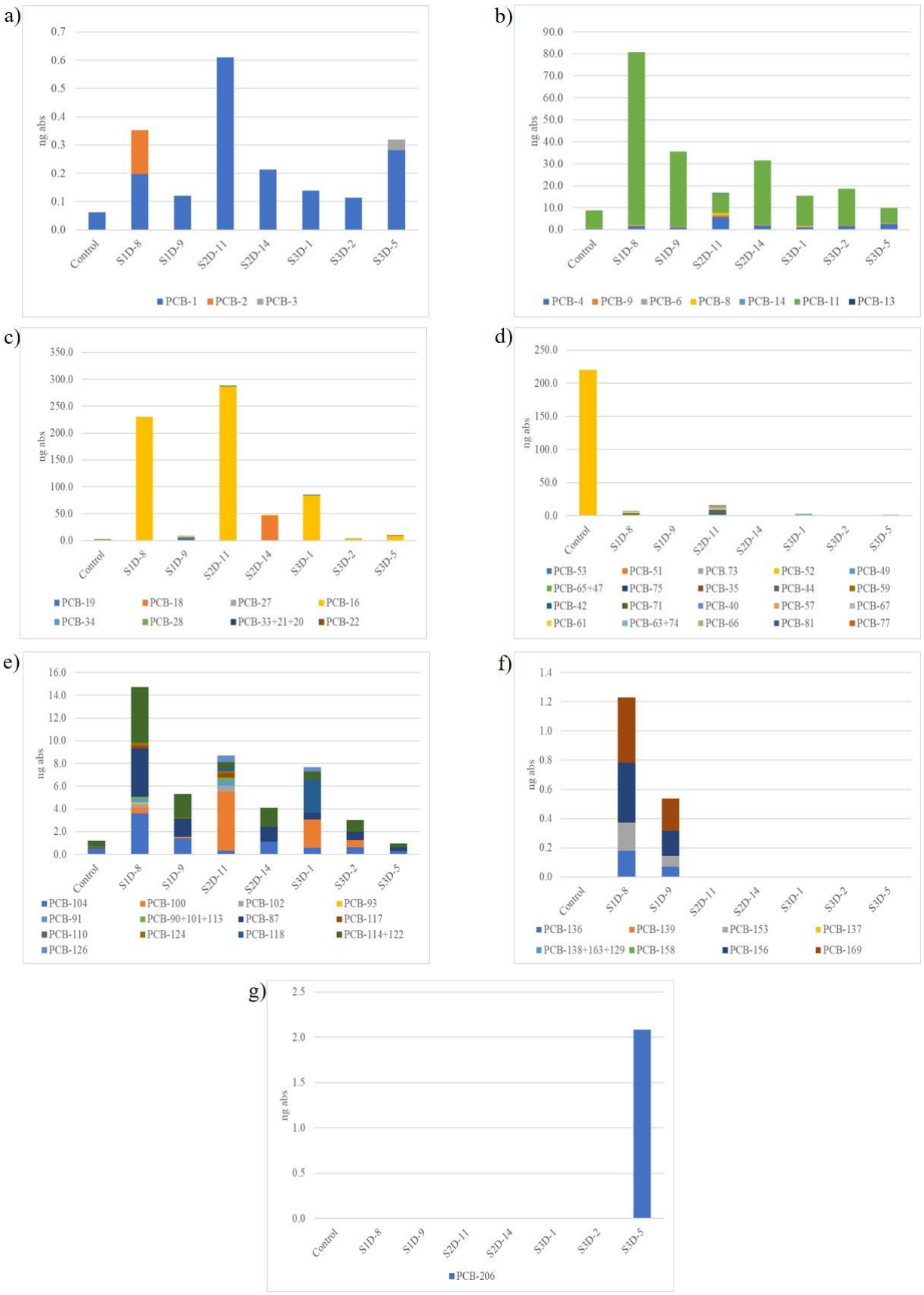


Fig. 17: PCB degradation rate based on different category of arctic strain. Particulary, the category are as follow: a) monobiphenyl, b) dibiphenyl, c) tribiphenyl, d) tetrabiphenyl, e) pentabiphenyl, f) hexabiphenyl and g) nonabiphenyl.

4.3.2 Antarctic lakes

4.3.2.1 Strain isolation

A total of 66 strains were isolated from the enrichments, distributed as follows: 14 from Lake Sofia, 16 from Argentina Lake, 8 from Crater Lake, 8 from Telefon Lake, and 12 from Ballaneros Lake. No strains were isolated from Extremadura Lake.

4.3.2.2 Test for polychlorinated biphenyl oxidation

Among these, 22 bacterial isolates were able to grow in the presence of Aroclor 1242. The *bph*A gene was detected in 15 of them (68%; Tab. 11), which were mainly identified as affiliates to the genus *Pseudomonas* (phylum Gammaproteobacteria). A single strain belonged to *P. antarctica*, two strains belonged to *P. fagi*, one to *P. gessani*, one to *P. helleri*, one to *P. lurida*, and one to *P. migulae*. Unfortunately, the affiliation could not be determined for seven strains (ASD-6, YASD-7, YAAD-3, ATD-2, ATD-3, ATD-5 and ATD-9). Nucleotide sequences have been deposited in the NCBI GenBank database (see Tab. 10).

Tab. 10: Identification of PCB-oxidant bacterial isolates by the 16S rRNA gene sequencing.

	Sample ID	Affilated organisms	Affiliation %	accession number	Phylum	Order	Family	Genus	Species
Zapatilla Lake (LZ)	AZD-1	*Not identified*							
	YAZD-5	*Not identified*							
Sofia Lake (LS)	ASD-3	*Pseudomonas fragi*	100	PQ686982	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*fragi*
	ASD-5	*Pseudomonas helleri*	99.53	PQ686985	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*helleri*
	ASD-6								
	ASD-8	*Pseudomonas fragi*	100	PQ686989	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*fragi*
	YASD-5	*Not identified*							
	YASD-7	*Not identified*							
Argentina Lake (LA)	YAAD-3	*Not identified*							
	YAAD-4	*Pseudomonas antarctica*	100	PQ686995	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*antarctica*
	YAAD-5	*Pseudomonas lurida*	100	PQ686998	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*lurida*
	YAAD-6	*Pseudomonas migulae*	99.7	PQ686977	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*migulae*
Telefon Lake (LT)	ATD-2	*Not identified*							
	ATD-3	*Not identified*							
	ATD-5	*Not identified*							
	ATD-8	*Pseudomonas gessardii*	100	PQ686988	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae	*Pseudomonas*	*gessardii*
	ATD-9	*Not identified*							

Tab. 11: Growth in the presence of Aroclor 1242 and presence of bphA gene, (+), weak response.

	ID strain	Positive test of	Presence of bph genes	Affilated organisms
Zapatilla Lake (LZ)	AZD-1	+	-	Not identified
	YAZD-5	(+)	-	Not identified
Sofia Lake (LS)	ASD-1	(+)	-	Not identified
	ASD-3	+	+	*Pseudomonas fragi*
	ASD-4	(+)	-	Not identified
	ASD-5	+	+	*Pseudomonas helleri*
	ASD-6	(+)	+	Not identified
	ASD-8	(+)	+	*Pseudomonas fragi*
	ASD-11	+	-	Not identified
	ASD-12	(+)	-	Not identified
	YASD-5	(+)	+	Not identified
	YASD-7	(+)	+	Not identified
Argentina Lake (LA)	YAAD-3	(+)	+	Not identified
	YAAD-4	(+)	+	*Pseudomonas antarctica*
	YASD-5	+	+	*Pseudomonas lurida*
	YASD-6	+	+	Pseudomonas migulae
Telefon Lake	ATD-2	+	+	Not identified
	ATD-3	+	+	Not identified
	ATD-5	+	+	Not identified
	ATD-8	(+)	+	Pseudomonas gessardii
	ATD-9	+	+	Not identified
	ATD-10	+	-	Not identified

4.3.2.3 PCB sequestration rate

Among positive strains, 7 were selected for PCB degradation tests on Aroclor 1242. In general, many strains showed high degradation efficiency, with YAAD-5 and ATD-2 that were the most efficient (Fig. 18) (both not identified).

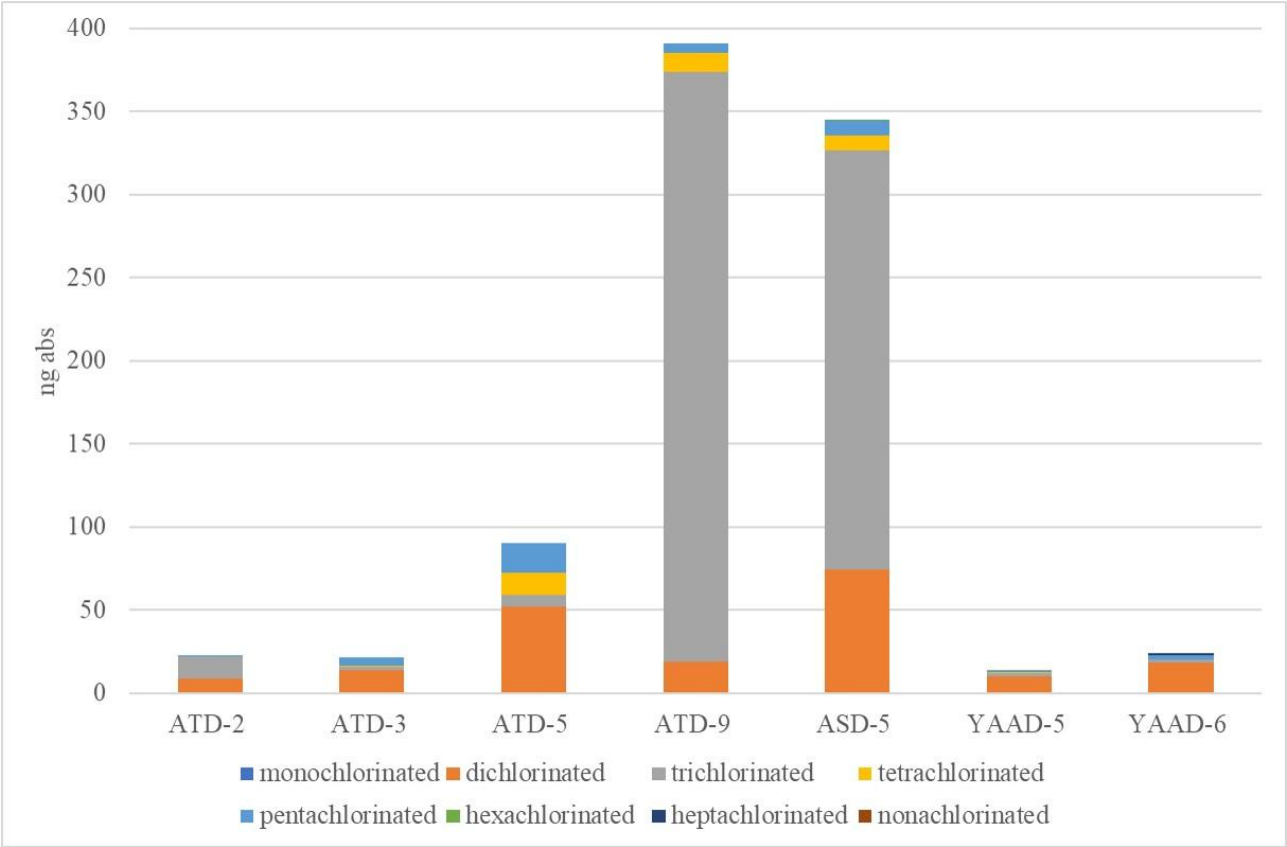


Fig. 18: PCB degradation rate of Antarctic bacterial strains.

Specifically, when analysing by category, a slight increase in monochlorinated compounds compared to the control was observed in all strains except for ATD-2, which showed values like the control; PCB-1 was the most prevalent compound in this category, present in all strains (Fig. 19a). Among dichlorinated compounds, some strains, such as ATD-5 and ASD-5, exhibited higher quantities than the control, while others showed values comparable to it, with PCB-11 being the most abundant molecule (Fig. 19b). For trichlorinated compounds, ATD-9 and ASD-5 displayed significantly higher values than the control, while other strains, including YAAD-5 (*Pseudomonas lurida*) and ATD-2, had values only slightly higher or comparable (Fig. 19c). In contrast, tetrachlorinated PCB were found below control levels in all strains, and in two cases ATD-2 and YAAD-6 (*Pseudomonas migulae*) they were not detected at all (Fig. 19d). For pentachlorinated PCB, ATD-2 and YAAD-5 (*Pseudomonas lurida*) showed values like the control, while the other strains had higher levels (Fig 19e). Finaly, small amounts of hexachlorinated compounds were detected in ASD-5 (*Pseudomonas*

helleri), and heptachlorinated compounds were identified in YAAD-6 (*Pseudomonas migulae*). All analysed strains demonstrated high efficiency in degrading tetrachlorinated PCBs, though some produced byproducts that increased the overall concentration of PCBs in the solution. ATD-2 and YAAD-5 (*Pseudomonas lurida*) emerged as the most effective strains across all PCB categories.

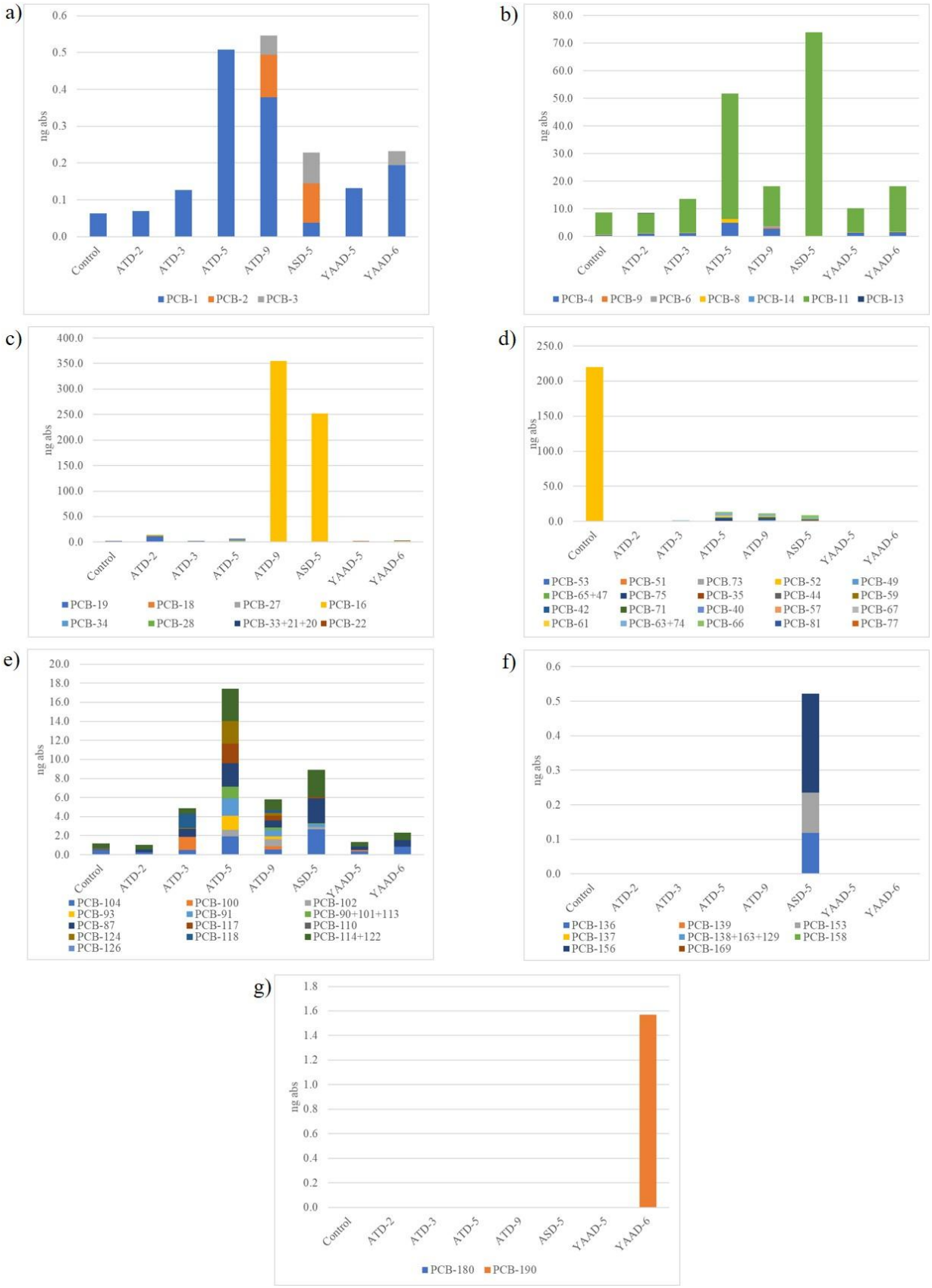


Fig. 19: PCB degradation rate based on different category of antarctic strain. Particulary, the category are as follow: a) monobiphenyl, b) dibiphenyl, c) tribiphenyl, d) tetrabiphenyl, e) pentabiphenyl, f) hexbiphenyl and g) heptabiphenyl.

4.3.3 Phylogenetic analysis of PCB strains

Overall, the phylogenetic tree (Fig. 20) included two groups, one containing the majority of strains and another with only two strains from Arctic lakes. In particular, in the first group were present all the strains were closely to the genus *Pseudomonas,* clusterizing in two subgroups. The first subgroup, included all the strains belonging to Antarctic lakes and six to Arctic lakes (S1D-11, S1D-8, S2D-5, S2D14, S3D-4 and S2D-6). Instead, the second subgroup evidenced only strains from Arctic lakes (S2D-6, S4D-8, S2D10, S4D-7, S2D-11, S1D-9, S4D-3, S4D-5 and S4D-6). The second group was instead divided into two subgroups, one close to the genus *Janthinobacterium* (including a single stain, S2D-8) and the other one closely related to the genus *Stenotrophomonas* (including a single stain, S1D-1).

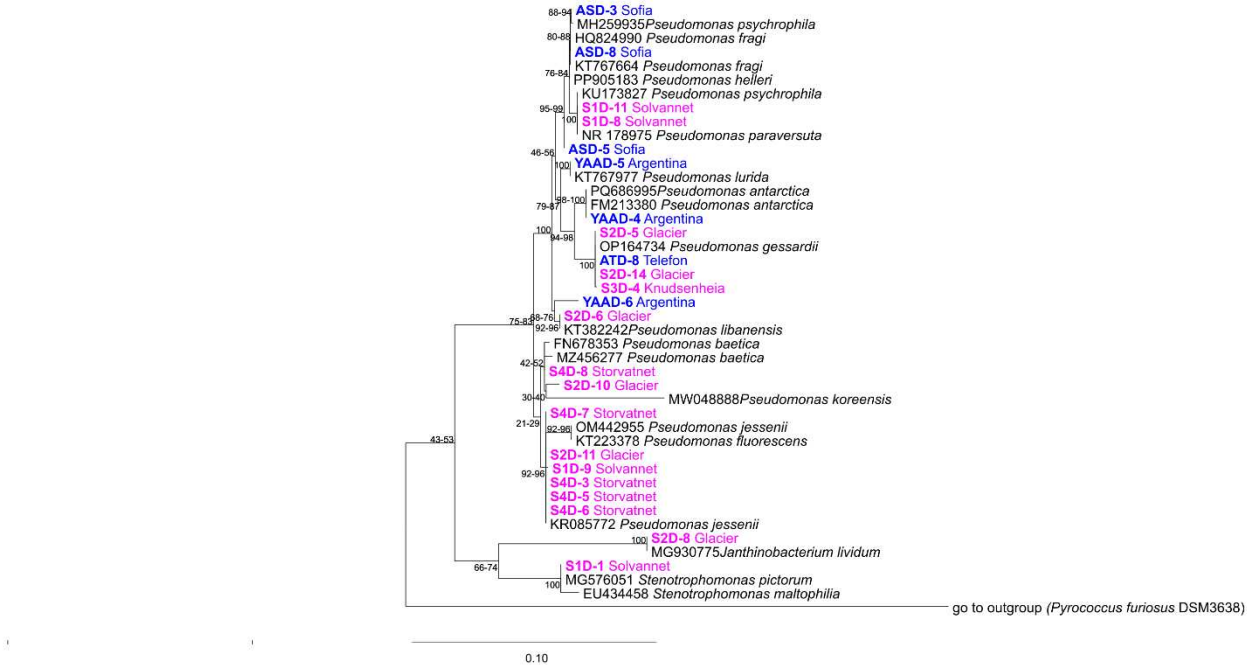


Fig. 20: Phylogenetic tree of PCB-oxidant bacterial isolates, (in pink Arctic strains; in bleu Antarctic strains).

4.3.4 Discussion

The evaluation of polychlorinated biphenyl (PCB) degradation in bacterial strains isolated from Arctic and Antarctic lakes showed significant results. Among the 46 strains tested from Arctic lakes,

24 (52%) demonstrated positive results for PCB degradation, with 18 of these strains possessing the *bphA* gene. In Antarctic samples, similarly, 22 out of 66 strains (33%) tested positive, with the *bphA* gene detected in 15 strains. This highlights a higher proportion of PCB-degrading strains in Arctic environments compared to Antarctic samples, which may reflect regional differences in environmental conditions and selective pressures. Strains such as S3D-2 and ATD-2 emerged as the most promising candidates for further study (Master & Mohn, 1998; Michaud et al., 2007; Weiland-Bräuer et al., 2017). These results were aligning with the work of Papale et al. (2022) on Antarctic lakes, indicating that aquatic habitats serve as ideal environments for the proliferation of PCB-degrading bacteria due to the accumulation of these persistent organic pollutants from both marine and terrestrial sources in the surrounding regions. The analysis of bacterial strains possessing the *bphA* gene revealed a predominance of the phylum Gammaproteobacteria, particularly within the genus *Pseudomonas*. Among the Arctic isolates, common species included *P. fluorescens*, *P. gessardii*, and *P. jessenii.* Similarly, Antarctic strains comprised *P. antarctica*, *P. fragi*, and *P. migulae*. The prevalence of these cold-adapted taxa has been previously documented (Bopp, 1986; Master & Mohn, 1998; Shuai et al., 2016) and underscores their potential for PCB biodegradation applications. PCB degradation assays revealed that Arctic strains generally demonstrated higher degradation efficiency across various PCB categories compared to Antarctic strains. Notably, Arctic strain S3D-2 and Antarctic strain ATD-2 (both affiliated with the genus *Pseudomonas*) emerged as the most effective across all categories. Analyzing specific PCB categories, dichlorinated and trichlorinated compounds exhibited varying degradation patterns. For instance, Arctic strains like S3D-2 showed efficient degradation of PCB-16, while Antarctic strain ATD-9 demonstrated partial degradation with notable byproduct formation. Tetrachlorinated compounds were universally well degraded, with Arctic strains such as S1D-9 and Antarctic strains like YAAD-5 showing near-complete removal. These findings align with the known capabilities of *Pseudomonas* to utilize biphenyl as a carbon source through the biphenyl dioxygenase pathway, encoded by the *bphA* gene, which initiates PCB breakdown (Bopp, 1986; Michaud et al., 2007; Furukawa & Fujihara, 2008).

Obtained results demonstrate the significant potential of cold-adapted bacteria, particularly those from the genus *Pseudomonas*, in degrading PCBs in polar environments. The potential of this cold-adapted taxa has been previously documented (Bopp, 1986; Master & Mohn, 1998; Shuai et al., 2016). The prevalence of the *bphA* gene and the associated metabolic pathways suggest that these strains are well-adapted to PCB degradation even under extreme conditions. Future studies should focus on characterizing the enzymatic pathways involved in PCB degradation, with particular attention to the *bphA* gene cluster and its role in biphenyl oxidation. Additionally, bioaugmentation strategies using these strains in PCB-contaminated polar environments could mitigate the environmental impact of PCB pollution. Given the "global distillation" phenomenon, which concentrates PCBs in polar regions due to their volatility and persistence (Furukawa & Fujihara, 2008), applying cold-adapted bacteria like *Pseudomonas* offer a promising avenue for bioremediation. Moreover, studies such as those by Papale et al. (2017), underline the importance of understanding biodiversity patterns and ecological interactions to inform effective PCB remediation approaches in extreme environments. Main results are part of a paper recently submitted to Polar Biology (Springer).

4.4 Correlation of results on the prokaryotic communities with chemical contamination

4.4.1 Preliminary analyses of retrieved metals in Arctic and Antarctic water

Preliminary data relatively to Antarctic water samples are reported in Tab. S1. Analyses of metals in Arctic lake water are still in progress. Retrieved metal concentrations across water samples of the Antarctic lakes revealed distinct patterns and variations. Telefon (LT), Extremadura (LE), and Ballanero Lakes (LB) exhibited the highest overall metal concentrations, with iron (Fe 56) and zinc (Zn 66) being the most abundant, reaching levels of 337.00 ppb and 483.00 ppb, respectively, in LE and LB. Copper (Cu 63) was another prominent metal, particularly in LB, where it peaked at 601.96 ppb, the highest among all lakes. In contrast, Argentina (LA), Sofia (LS), and Zapatilla Lakes (LZ) displayed much lower overall metal concentrations, with zinc being the most represented metal in these lakes (8.33 ppb in LA and 9.85 ppb in LS). Vanadium (V 51) showed a notable peak in Crater Lake at 230.73 ppb, significantly higher than in other lakes, where it remained minimal. Thorium (Th 232), uranium (U 238), and thallium (Tl 205) were consistently among the least represented metals across all lakes, with concentrations often below 1 ppb.

4.4.2 Chemical organic compounds retrieved across Arctic and Antarctic lake water and sediment

Persistent organic pollutant distribution across all analyzed lakes are reported in Fig. 21 and in Tab. S2. As it is shown in Fig. 21a, there was a clear separation between water and sediments in Arctic lakes about chlorobenzene (CB). It can also be observed that the water in Arctic lakes has a stronger correlation with the lighter component of this group (i.e., Monochlorobenzene, Dichlorobenzene, 1,2), the sediments in Solvannet (L1) and Glacier Lakes (L2) were correlated with Hexachlorobenzene and 1,2,3,4 Tetrachlorobenzene. While, sediments of Knudsenheia (L3), Storvatnet (L4), and Tvillingvatnet Lakes (L5) showed a stronger correlation with

Pentachlorobenzene, 1,2,4,5, Tetrachlorobenzene , 1,3,5, Trichlorobenzene and ∑1,2,4,5 + 1,2,3,5 Tetrachlorobenzene. For Antarctica (Fig 21e), there was also a clear separation between water and sediments correlated with CB. Similarly, the lighter components were correlated with water, while the heavier ones correlated with sediments. Specifically, the water from Crater (LC), Zapatilla (LZ), Sofia (LS), Argentina (LA), and Telefon Lakes (LT) correlated with Monochlorobenzene, while Extremadura (LE) and Ballaneros Lakes (LB) correlate with 1,2, Dichlorobenzene, 1,3, Dichlorobenzene , 1,4, Dichlorobenzene, and 1,2,4 Trichlorobenzene. For the sediments, Zapatilla (LZ), Extremadura (LE), Crater (LC), and Ballaneros Lakes (LB) correlate with Trichlorobenzene 1,2,3, Pentachlorobenzene, and Hexachlorobenzene, whereas Telefon Lake (LT) correlated with 1,3,5 Trichlorobenzene. It is also notable that the sediments from Sofia (LS) and Argentina Lakes (LA), as well as the water from Ballaneros Lake (LB), are located far from the all the other samples.

Regarding Arctic polycyclic aromatic hydrocarbons (PAHs) (Fig. 21b), water and sediments were also separated. Notably sediments from Solvannet Lake (L1) showed a strict domination in the analyses while correlated with Phenanthrene, Benzo(b)fluoranthene, Anthracene, Benzo(a)anthracene, Chrysene, Benzo(k)fluoranthene, Perylene, Fluorene, and Fluoranthene. Tvillingvatnet Lake (L5) correlates with Naphthalene, Acenaphthene, Dibenzo(a,h)anthracene, Benzo(g,h,i)perylene, Acenaphthylene, Naphthalene-2-methyl, Benzo(a)pyrene, and Pyrene.

In Antarctic samples, there was also a separation between sediments and water (Fig. 21f). Specifically, the water from Sofia (LS) and Argentina Lakes (LA) correlates with Phenanthrene, Anthracene, Naphthalene, Acenaphthylene, Naphthalene-2-methyl, and Pyrene, water of Telefon, Zapatilla, and Crater Lakes (LC) correlate with Fluorene, Benzo(e)pyrene, Perylene, Dibenz(a,h)anthracene, Benzo(a)pyrene, and Indeno(1,2,3-cd)pyrene. Sediments from Ballaneros Lake (LB) correlate with Fluoranthene, while Telefon (LT), Extremadura (LE), Zapatilla (LZ), Crater (LC), Sofia (LS), and Argentina Lakes (LS) correlate with Benz(a)anthracene, Chrysene, and Acenaphthene.

Regarding Polychlorinated naphthalenes (PCNs) in Arctic lakes (Fig. 21c), a clear distinction was observed between water and sediments. Specifically, the water from Glacier (L2), Storvatnet (L4), and Tvillingvatnet Lakes (L5) correlates with 1,2,3,5,7 Pentachloronaphthalene, 2 Monochloronaphthalene, and 1,5 Dichloronaphthalene, while the water from Solvannet (L1) and Knudsenheia Lakes (L3) correlates with 2,3,6,7 Tetrachloronaphthalene, 1,2,3, Trichloronaphthalene, and Octachloronaphthalene. However, sediments showed a unique group with no appreciable correlated with any PAHs.

In Antarctic lakes (Fig. 21g), the analysis showed the same separation between sediments and water. Specifically, the water from Sofia (LS), Argentina (LA), Zapatilla (LZ), Telefon (LT), and Crater Lakes (LC) correlates with 1,2,3 Trichloronaphthalene and 1,5 Dichloronaphthalene, while the water from Extremadura (LE) and Ballaneros Lakes (LB) shows correlations with 2,3,6,7 Tetrachloronaphthalene, 1,2,3,4 Tetrachloronaphthalene, 1,2,3,5,7,8 Hexachloronaphthalene, and 1,2,3,4,6 Pentachloronaphthalene. Sediments from Sofia (LS), Argentina (LA), and Telefon Lakes (LT) correlate with ∑1,2,3,5 + 1,2,5,6 Tetrachloronaphthalene and 1,2,3,5,8 Pentachloronaphthalene, while Ballaneros (LB), Crater (LC), Extremadura (LE), and Zapatilla Lakes (LZ) correlate with 2-Monochloronaphthalene and Octachloronaphthalene.

Finally, for Arctic PCBs, there is no sharp division between water and sediments as observed in other cases (Fig. 21d). Specifically, the water from Solvannet (L1), Storvatnet (L4), and Tvillingvatnet Lakes (L5) correlates with PCB 81, whereas the sediments from Solvannet Lake (L1) correlate with PCB 99, PCB 151, PCB 77, PCB 123, PCB 74, PCB 149, and ∑PCB 84 + PCB 90 + PCB 101. The sediments from Knudsenheia (L3) and Tvillingvatnet Lakes (L5) correlate with PCB 52, ∑PCB 31+28, PCB 110, ∑PCB 84 + PCB 90 + PCB 101, ∑PCB 153 + PCB 168, and PCB 118. In Antarctic samples across PCBs (Fig. 19h), the separation between water and sediments was observed strongly. Specifically, the water from Extremadura (LE), Ballaneros (LB), and Telefon Lakes (LT) correlates with PCB 123, PCB 151, PCB 99, PCB 77, ∑PCB 31 + PCB 28, PCB 105, and PCB 81, while no

correlations were found for the water from Crater (LC), Zapatilla (LZ), Argentina (LA), and Sofia Lakes (LS). The sediments from Sofia (LS), Argentina (LA), Telefon (LT), and Ballaneros Lakes (LB) correlate with ∑PCB 138 + PCB 158, PCB 149, PCB 74, PCB 52, PCB 110, PCB 118, ∑PCB 155 + PCB 168, and ∑PCB 84 + PCB 90 + PCB 101.

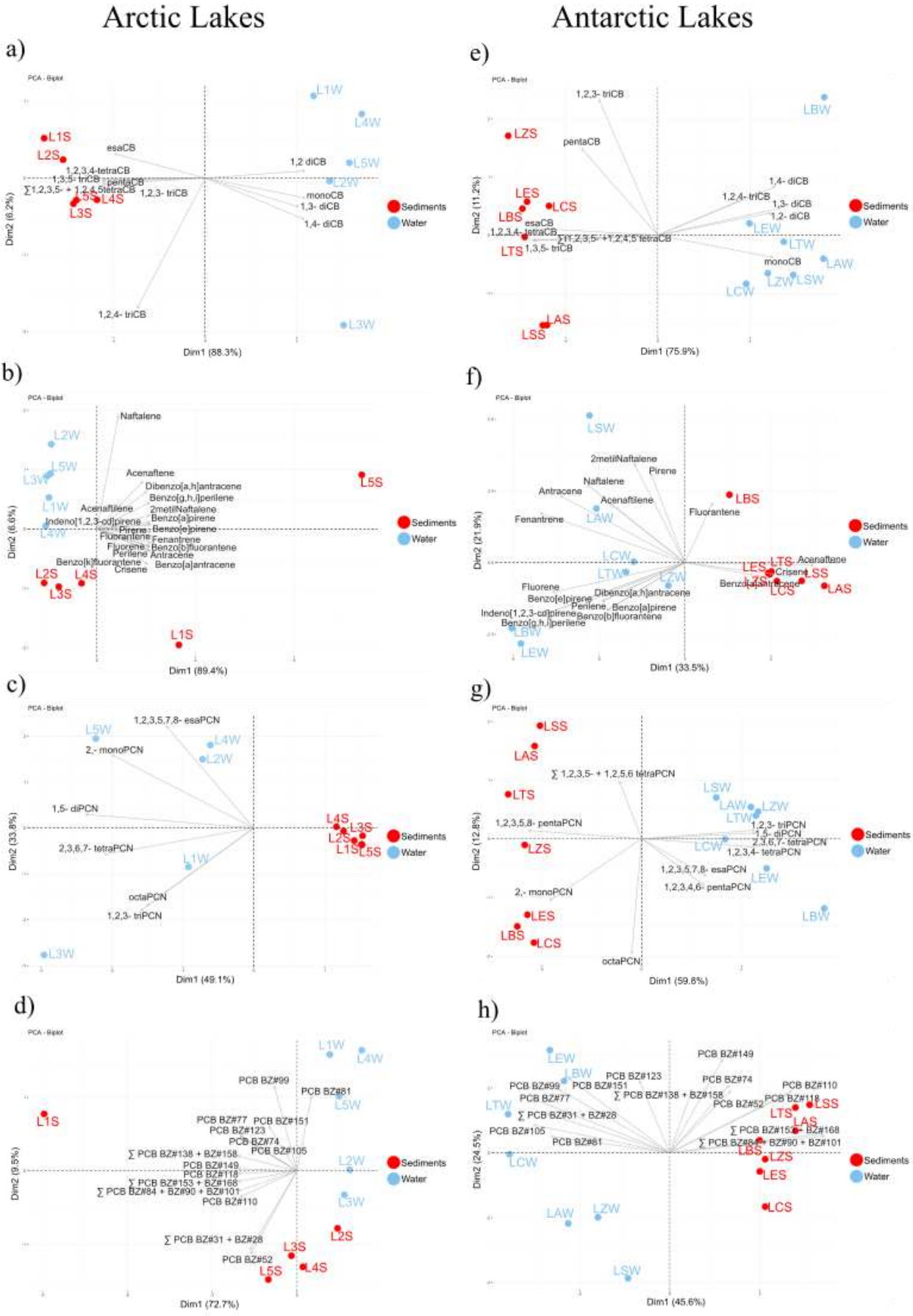

Arctic Lakes
Antarctic Lakes
a)
e)
b)
f)
c)
g)
d)
h)
PCA - Biplot
Dim1 (88.3%)
Dim2 (6.2%)
Dim1 (75.9%)
Dim2 (11.2%)
Dim1 (89.4%)
Dim2 (6.6%)
Dim1 (33.5%)
Dim2 (21.9%)
Dim1 (49.1%)
Dim2 (33.8%)
Dim1 (59.6%)
Dim2 (12.8%)
Dim1 (72.7%)
Dim2 (9.5%)
Dim1 (45.6%)
Dim2 (24.5%)
Sediments
Water
L1W
L4W
L1S
L2S
esaCB
1,2,3,4-tetraCB
1,3,5- triCB
pentaCB
∑1,2,3,5- + 1,2,4,5tetraCB
1,2,3- triCB
L5S
L4S
L3S
1,2 diCB
L5W
L2W
monoCB
1,3- diCB
1,4- diCB
1,2,4- triCB
L3W
1,2,3- triCB
LBW
LZS
pentaCB
LES
LCS
LBS
esaCB
1,2,3,4- tetraCB
∑1,2,3,5- +1,2,4,5 tetraCB
LTS
1,3,5- triCB
1,4- diCB
1,2,4- triCB
1,3- diCB
1,2- diCB
LEW
LTW
monoCB
LAW
LZW
LSW
LCW
LAS
LSS
Naftalene
L2W
L5W
L3W
Acenaftene
Dibenzo[a,h]antracene
Benzo[g,h,i]perilene
L5S
L1W
Acenaftilene
2metilNaftalene
Indeno[1,2,3-cd]pirene
Benzo[a]pirene
Pirene
Benzo[e]pirene
L4W
Fluorantene
Fenantrene
Fluorene
Benzo[b]fluorantene
Perilene
Antracene
Benzo[k]fluorantene
Benzo[a]antracene
Crisene
L2S
L4S
L3S
L1S
LSW
2metilNaftalene
Pirene
Naftalene
Antracene
Acenaftilene
LBS
Fluorantene
LAW
Fenantrene
LCW
LTW
LZW
LES
LTS
Acenaftene
Crisene
LSS
LZS
LCS
LAS
Fluorene
Benzo[e]pirene
Dibenzo[a,h]antracene
Perilene
Benzo[a]pirene
Indeno[1,2,3-cd]pirene
Benzo[b]fluorantene
Benzo[g,h,i]perilene
LBW
LEW
1,2,3,5,7,8- esaPCN
L5W
2,- monoPCN
L4W
L2W
1,5- diPCN
L4S
L3S
L2S
L1S
L5S
2,3,6,7- tetraPCN
L1W
octaPCN
1,2,3- triPCN
L3W
LSS
LAS
∑ 1,2,3,5- + 1,2,5,6 tetraPCN
LTS
LSW
LAW
LZW
LTW
1,2,3,5,8- pentaPCN
1,2,3- triPCN
1,5- diPCN
2,3,6,7- tetraPCN
LZS
LCW
1,2,3,4- tetraPCN
1,2,3,5,7,8- esaPCN
LEW
1,2,3,4,6- pentaPCN
2,- monoPCN
LBW
LES
LBS
LCS
octaPCN
L1S
PCB BZ#99
PCB BZ#81
L5W
PCB BZ#77
PCB BZ#151
PCB BZ#123
PCB BZ#74
∑ PCB BZ#138 + BZ#158
PCB BZ#105
L2W
PCB BZ#149
PCB BZ#118
∑ PCB BZ#153 + BZ#168
∑ PCB BZ#84 + BZ#90 + BZ#101
PCB BZ#110
L3W
∑ PCB BZ#31 + BZ#28
L2S
L3S
PCB BZ#52
L5S
L4S
LEW
LBW
PCB BZ#149
PCB BZ#123
PCB BZ#74
PCB BZ#110
PCB BZ#99
PCB BZ#151
∑ PCB BZ#138 + BZ#158
PCB BZ#77
PCB BZ#118
LSS
PCB BZ#52
LTW
∑ PCB BZ#31 + BZ#28
LTS
LAS
PCB BZ#105
∑ PCB BZ#153 + BZ#168
PCB BZ#81
∑ PCB BZ#84 + BZ#90 + BZ#101
LBS
LZS
LCW
LES
LAW
LZW
LCS
LSW

Fig. 21: PCAs performed across all analyzed Lakes and all analysed Persistent organic pollutants. Particularly a) Cloro benzene (CB) in Arctic lakes; b) Polycyclic aromatic hydrocarbons (PAH) in Arctic lakes; c) Polychlorinated naphthalenes (PCNs) in Arctic lakes; d) Polychlorinated biphenyls (PCB) in Arctic lakes; e) Cloro benzene (CB) in Antarctic lakes; f) Polycyclic aromatic hydrocarbons (PAH) in Antarctic lakes; g) Polychlorinated naphthalenes (PCNs) in Antarctic lakes; h) Polychlorinated biphenyls (PCB) in Antarctic lakes.

4.4.3 Comparison of Arctic and Antarctic lakes across retrieved organic chemicals

PCA comparing results of the POPs retrieved across all lakes in Arctic and Antarctic areas was performed and the results are shown in Fig. 22. Fig. 22a shown that in the case of CBs, both sediments and water of Arctic and Antarctic lakes were separated from each other. Additionally, the sediments and water are grouped by type. Specifically, in the first quadrant, we found only the sediments of Arctic lakes; in the second quadrant, we found the water of Antarctic lakes, except for the water of Crater Lake, which is in the third quadrant but very close to the others. In the third quadrant, we find the water of Arctic lakes, and finally, in the fourth quadrant, we found all the sediments of Antarctic lakes (Fig. 22a). Antarctic water evidenced a relationship with di- and tri-CBs, while Arctic water was mostly associated with mono-CB. In the same way, tetra- penta- and esa-CB were mostly related to Arctic sediments while Antarctic.

Regarding PAHs, it can be observed that there was no clear separation between the two poles or between the matrices. Only a few samples deviated from the groupings, including the water of Sofia (LS), Argentina (LA), and Ballaneros Lakes (LB). The sediments of Solvannet (L1) and Tvillingvatnet Lakes (L5) were in the fourth and first quadrants, respectively (Fig. 22b).

For PCNs, the graph showed that the two poles are divided. All the sediments and water of Arctic lakes are found in the first quadrant, except for the water of Knudsenheia Lake (L3), which was in the second quadrant along with the water of Zapatilla (LZ) and Sofia Lakes (LS). Meanwhile, the water of Antarctic lakes, including Argentina (LA), Ballaneros (LB), Extremadura (LE), Crater (LC), and Telefon (LT), were all found in the third quadrant. Lastly, the sediments of Antarctic lakes wase all in the fourth quadrant (Fig. 22c).

For PCBs, the graph highlights that the sediment of Solvannet Lake (L1) is separate from the others and that there is no clear separation between the two poles or between the matrices. Specifically, in the first quadrant, we found the water samples of Arctic lakes Knudsenheia (L3), Storvatnet (L4), Tvillingvatnet (L5), and both the water and sediment of Glacier Lake (L2). Near this grouping but in the fourth quadrant, we found the water of Sofia Lake (LS) and Solvannet Lake (L1). Additionally, in the first quadrant, we also found the Arctic sediments of Storvatnet (L4) and Knudsenheia Lakes (L3), along with the sediment of Crater Lake (LC) in Antarctica. Close to this group but in the second quadrant was the sediment of Tvillingvatnet Lake (L5), as well as the remaining sediments of Antarctic lakes. In the third quadrant, we found the water of Antarctic Ballaneros (LB), Extremadura (LE), Telefon (LT), and Crater Lakes (LC). Finally, in the fourth quadrant, there was the water of Antarctic Zapatilla (LZ) and Argentina Lakes (LA), which form a group together (Fig. 22d).

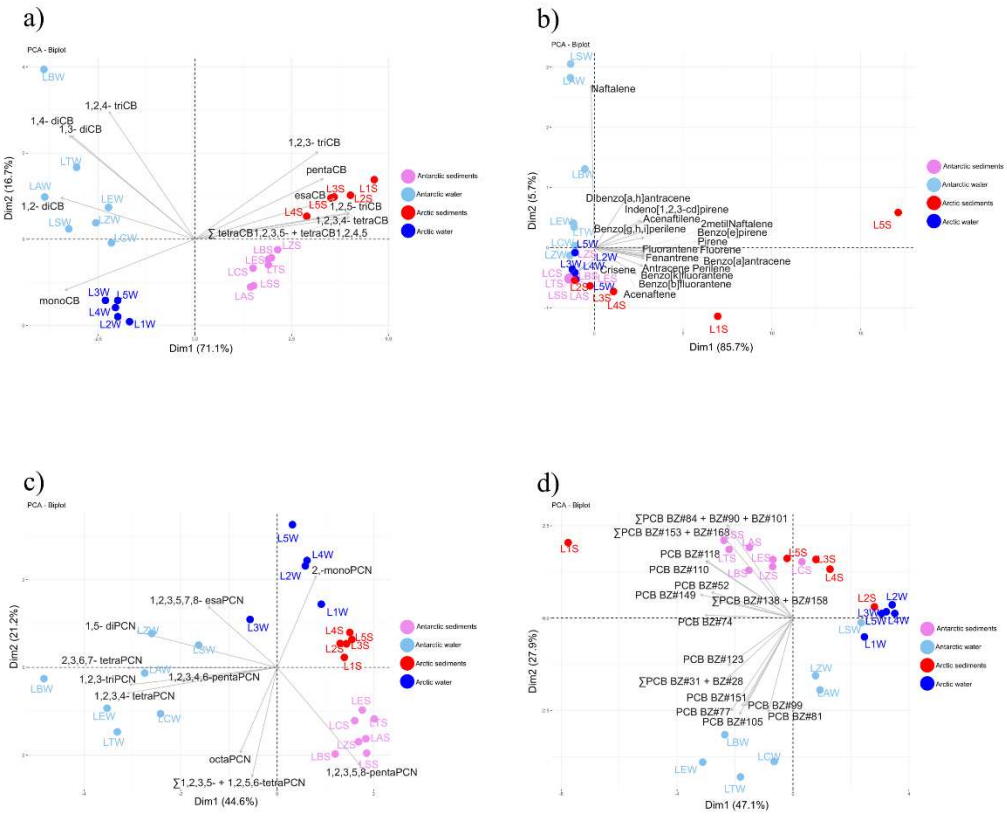


Fig. 22: PCAs performed across all analyzed Lakes and all analysed Persistent organic pollutants. Particularly a) Cloro benzene (CB) in Arctic and Antarctic lakes; b) Polycyclic aromatic hydrocarbons (PAH) in Arctic and Antarctic lakes; c) Polychlorinated naphthalenes (PCNs) in Arctic and Antarctic lakes; d) Polychlorinated biphenyls (PCB) in Arctic and Antarctic lakes.

4.4.4 Comparison of microbial communities and chemical compounds

4.4.4.1 Total prokaryotic community vs retrieved metals

The relationship between total microbial communities and analyzed metals was evaluated using Spearman's correlation matrix (Fig. 23) only across water Antarctic samples. The figure illustrated significant correlations (p-value < 0.05) between the most abundant bacterial phyla and metals retrieved. Campylobacteriota and Desulfobacteriota showed strong positive correlations with almost all retrieved metals. In particular, Campylobacteriota showed no relation only with vanadium (V) and arsenic (As). Similarly, Desulfobacteriota showed no relation with vanadium (V) and arsenic (As) but also with uranium (U). Conversely, Firmicutes and Alphaproteobcteria showed few relations with analyzed metals. Firmicutes highlighted a strong positive relation with copper (Cu) and zinc (Zn), while Alphaproteobacteria were positively correlated with arsenic (As).

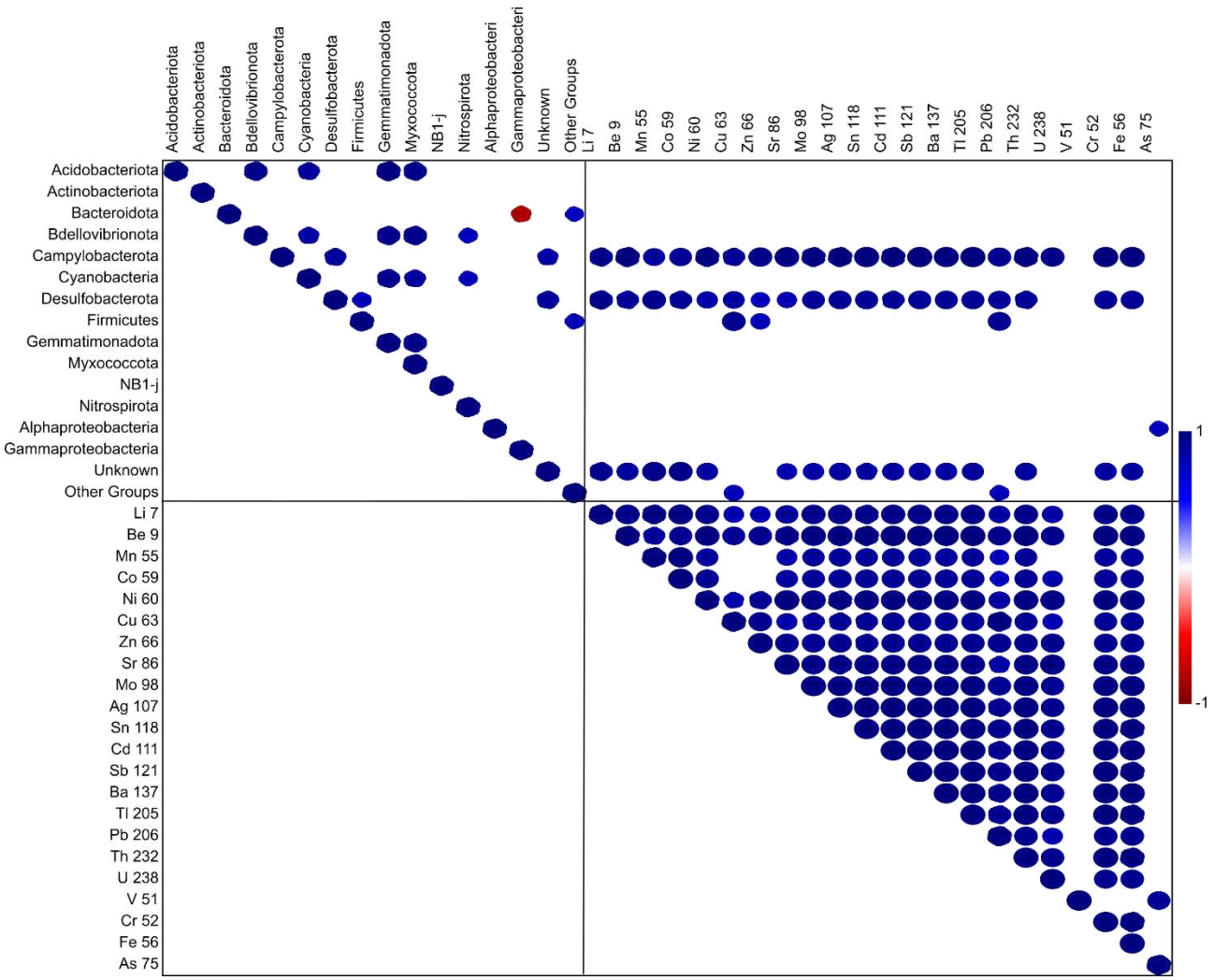


Fig. 23: Spearman correlation matrix. In the figure are reported only dots that represent statistically correlations (p < 0.05), whereas the dimensions are inverse to the strongest of correlation (bigger are the dots minor is the p).

4.4.4.2 Total prokaryotic community vs persistent organic pollutants

The relationship among total microbial communities and POPs was evaluated using Spearman's correlation matrix (Fig. 24). The figure illustrated significant correlations (p-value < 0.05) between the most abundant bacterial phyla and POPs retrieved across all samples. Acidobacteriota and Actinobacteriota showed strong negative correlations with ΣPAHs, ΣPCBs, and higher-substituted POPs like tetra-, penta-, and octa-substituted PCNs and CBs, suggesting sensitivity to these compounds. In contrast, Gammaproteobacteria demonstrated extensive positive correlations across all pollutant categories, including mono-, di-, tri-substituted CBs and PCNs, as well as ΣPAHs and ΣPCBs, indicating its broad association with both low- and high-substituted POPs. Cyanobacteria

were positively correlated with ΣPAHs, ΣPCBs, and higher-substituted POPs, reflecting their adaptability to environments with significant pollutant loads. Firmicutes and Desulfobacterota displayed positive correlations with lower-substituted CBs and PCNs, particularly mono-, di-, and tri-substituted compounds, but their correlations with higher-substituted POPs were less pronounced. Bacteroidota showed mixed correlations, with positive associations for lower-substituted POPs but negative correlations with tetra- and penta-substituted compounds. Myxococcota and Nitrospirota showed positive correlations with ΣPAHs and tetra-substituted CBs and PCNs, while Gemmatimonadota and Bdellovibrionota were associated primarily with lower-substituted CBs and PCNs. Alphaproteobacteria evidenced positive correlations with mono-, di-, and tri-substituted compounds but negative correlations with higher-substituted POPs. Overall, the data highlighted a trend where lower-substituted POPs (e.g., mono-, di-, tri-substituted CBs and PCNs) exhibited positive correlations across a broader range of bacterial phyla, while higher-substituted POPs (e.g., tetra-, penta-, octa-substituted compounds) were more strongly associated with specific phyla, such as Gammaproteobacteria and Cyanobacteria, and negatively correlated with others like Actinobacteriota and Bacteroidota.

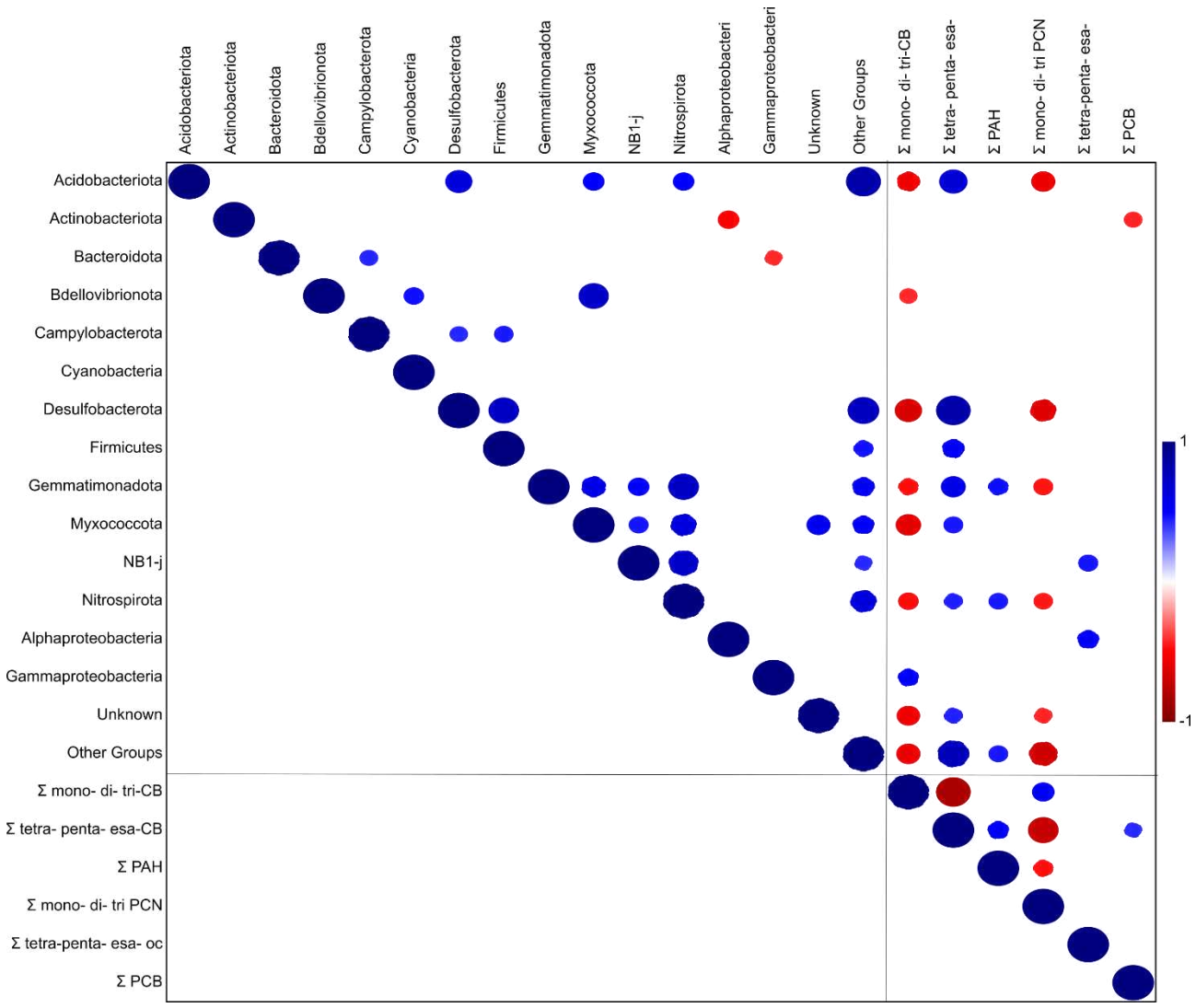


Fig. 24: Spearman correlation matrix. In the figure are reported only dots that represent statistical correlations ($p < 0.05$), whereas the dimensions are inverse to the strongest of correlation (bigger are the dots minor is the p).

4.4.5 Discussion

The concentrations of retrieved chemicals across Arctic and Antarctic lakes reveal distinct patterns and variations, with notable differences and similarities between lakes and regions. The Antarctic analyzed lakes, in particular, Telefon (LT), Extremadura (LE), and Ballaneros (LB), exhibited higher overall concentrations of metals, with zinc (Zn) and iron (Fe) being the most abundant, peaking at 483.00 ppb and 337.00 ppb, respectively. Copper (Cu) also showed significant concentrations, particularly in Ballaneros Lake (LB) (601.96 ppb). In contrast, Argentina (LA) and Sofia Lakes (LS) had much lower overall metal concentrations, with only moderate zinc levels (8.33–9.85 ppb). The

high metal concentrations in some Antarctic lakes can be attributed to various factors, including geologic influences, atmospheric deposition, and potential anthropogenic sources (Neethu et al., 2015; Davidson et al., 2003).

The Arctic lakes displayed a different chemical profile, with a more significant presence of persistent organic pollutants like polychlorinated biphenyls. These compounds can originate from long-range atmospheric transport and deposition, as well as remobilization from melting glaciers (Blais et al., 2001; Davidson et al., 2003). Arctic lakes such as Solvannet (L1) and Glacier Lakes (L2) had higher chlorobenzenes (CBs) levels in sediments, particularly heavier compounds like tetrachlorobenzene and hexachlorobenzene. At the same time, lighter CBs, such as monochlorobenzene and dichlorobenzene, dominated the water samples. This distribution pattern suggests the potential for cold condensation and selective sequestration of more recalcitrant POPs in Arctic environments (Malmquist et al., 2003). In comparison, the Antarctic Lakes exhibited relatively low levels of these persistent organic pollutants. Comparing Arctic and Antarctic ecosystems, it is evident that while both regions accumulate contaminants, the specific chemical profiles differ depending on factors such as long-range transport pathways, local sources, and environmental conditions (Blais et al., 2001; Davidson et al., 2003; Malmquist et al., 2003).

Analyzing the retrieved microbial communities' correlations with the retrieved chemicals, results showed potential specialization or sensitivity. For metals, Antarctic microbial phyla such as Campylobacteriota and Desulfobacteriota exhibited strong positive correlations with most analyzed metals, suggesting adaptability to metal-enriched environments. In contrast, specific phyla like Firmicutes and Acidobacteriota showed few positive associations with metal concentrations, implying potential sensitivity or specialization to tolerate specific metals (Corcoll et al., 2018; Chen et al., 2019). About persistent organic pollutants, retrieved phyla like Acidobacteriota, Desulfobacteriota, Firmicutes, Gemmatimonadota, Mixococcota and Nitrospirota showed positive associations with lower-substituted CBs, while negative correlation with higher-substituted CBs.

Indicating the potential adaptability of these phyla to handle certain lipophilic persistent organic compounds, potentially through transformation or sequestration (Kannan et al., 1995; Cerro-Gálvez et al., 2019;). Regarding retrieved PAH concentrations, only Gemmatimonadota and Nitrospirae displayed strong positive correlations, suggesting these microbes may be involved in the cycling and transformation of these complex (Hassanshahian et al., 2015). Conversely, PCB concentrations showed no correlation with retrieved phyla except for a negative correlation with Actinobacteriota, confirming their recalcitrant and difficult oxidation patterns (Petrić et al., 2011).

Finally, the analysis of PCN highlighted a positive correlation between Alphaproteobacteria and NB1-j group with higher-substituted PCN. In contrast, a negative correlation was shown between Desulfobacteriota, Gemmatimonadota, and Nitrospirota with the lower-substituted PCN, indicating also, in this case, a potential selective adaptation of these phyla to handle the more complex persistent organic pollutants (Maksimov et al., 2019). These results underscore the role of local environmental factors, such as temperature and organic matter content, in shaping pollutant distribution and interactions with microbial communities. In general, the retrieved chemical profiles, including metals, persistent organic pollutants, and polycyclic aromatic hydrocarbons, exhibited distinct regional patterns correlated with the observed differences in microbial community composition across the Arctic and Antarctic lakes systems (Blais et al., 2001; Davidson et al., 2003; Malmquist et al., 200).

5 Conclusions

Results obtained in this Ph.D. Thesis deal with several microbial aspects concerning the Arctic and Antarctic lakes under examination, from the study of bacterial communities in water and sediment to the isolation of bacterial strains capable of tolerating heavy metals or degrading PCBs, throughout the response of bacterial community to environmental pollutants (i.e., metals and POPs). The investigation on the microbial community structure and composition highlights a high similarity at phylum level between Arctic and Antarctic lakes. However, differences became evident when water

and sediment samples were compared, particularly in lakes with similar physicochemical profiles. It is noteworthy that differences in community composition at family and genus levels were retrieved, suggesting that geographic distance and the physico-chemical characteristics of the lakes influence microbial communities. The analyses of heavy metal enrichments of sediment samples demonstrated a high tolerance to iron, common at both poles, followed by mercury and copper. Multitolerance testing selected 25 strains (15 from the Arctic and 10 from Antarctica) showing a higher tolerance towards mercury than copper. Furthermore, differences between the poles emerged concerning bacterial isolate identification. Most Arctic strains belonged to the phylum Gammaproteobacteria, predominantly in the genus *Pseudomonas*. In contrast, Antarctic strains primarily belonged to the phylum Actinobacteriota, with genera such as *Subercola*, *Anthrobacter*, and *Pseudoarthrobacter* being the most represented. Finally, the sequestration rates for the two tested metals (iron and mercury) showed a low efficiency. The Antarctic species *Arthrobacter livingstonensis* displayed an efficiency of 14% in iron sequestration, while the Arctic strain *Pseudomonas extremaustralis* exhibited a 10% rate for mercury tolerance. Bacteria isolated from biphenyl enrichments of sediment samples demonstrated a high efficiency in PCB (namely Aroclor 1242) degradation in both poles. More strains tested positive for PCB oxidation in the Arctic than in Antarctica (52 and 33%, respectively). The identification of PCB-oxidising bacterial strains revealed that most of them belonged to the genus *Pseudomonas* (Gammaproteobacteria). Moreover, the PCB degradation test showed that all tested strains were capable of PCB degradation, especially of tetrachlorinated compounds, with some strains from both poles achieving a complete degradation. The prevalence of the *bphA* gene and associated metabolic pathways suggests that these strains are well-adapted to PCB degradation, even under extreme conditions in remote areas of the globe. Additionally, the chemical concentrations found in the Arctic and Antarctica revealed distinct patterns, with differences and similarities among lakes and regions. Metal concentrations were generally higher in Antarctica, particularly in the brackish lakes Telefon, Extremadura, and Ballaneros, where elevated levels of zinc and iron were recorded. Conversely, POPs were predominantly detected in Arctic lakes, with

differences noted between water and sediment: heavier compounds were found in sediments, while lighter compounds were present in water. Concerning the correlation of pollutants with bacterial phyla, heavy metals were positively correlated with Campylobacterota and Desulfobacterota. POPs, on the other hand, showed stronger correlations with the lighter components of CBs, whereas PCBs did not exhibit any correlation. The study findings reveal diverse microbial communities across the Arctic and Antarctic regions, as well as in water and sediment samples, suggesting that geographic distance and the physicochemical characteristics of the lakes may shape inhabiting microbial assemblages. The high biotechnological potential of the polar bacterial isolates, particularly against PCBs, underscores the vital role of microbial communities in remediating these pollutants. These findings emphasize the influence of geographic and physicochemical factors in shaping polar microbial communities and their biotechnological potential for pollutant remediation. Future research should focus on genus-level characterizations, particularly of unknown taxa in sediments, detailed analyses of genetic pathways underlying heavy metal resistance, and optimization of co-culture systems to improve pollutant sequestration. Further investigation into the enzymatic pathways, especially the *bphA* gene cluster and its role in biphenyl oxidation, could enhance our understanding of PCB degradation. This study demonstrates the crucial role of microbial communities in polar ecosystems and their potential for addressing environmental challenges, paving the way for more targeted and effective biotechnological applications.

6 Supplementary Material

Tab. S1: Metals retrieved concentrations in Antarctic water lakes

Sample Id	Argentina Lake (LA)	Sofia Lake (LS)	Zapatilla Lake (LZ)	Crater Lake (LC)	Telefon Lake (LT)	Extremadura Lake (LE)	Ballanero Lake (LB)
Li 7(ppb)	0.28	0.14	0.34	8.79	217.48	143.71	145.92
Be 9(ppb)	0.03	0.03	0.03	0.03	0.60	0.60	0.60
Mn 55(ppb)	1.09	1.00	0.50	1.40	94.78	46.00	46.00
Co 59(ppb)	0.02	0.02	0.01	0.04	2.51	1.52	1.20
Ni 60(ppb)	0.23	0.17	0.10	0.30	15.53	18.05	12.57
Cu 63(ppb)	1.21	1.57	0.52	3.93	291.00	328.68	601.96
Zn 66(ppb)	8.33	9.85	5.00	15.00	241.99	483.00	483.00
Sr 86(ppb)	10.80	1.63	3.24	28.58	4903.41	6958.63	4628.30
Mo 98(ppb)	0.24	0.23	0.14	3.49	15.00	15.00	15.00
Ag 107(ppb)	0.04	0.04	0.02	0.07	2.20	2.20	2.20
Sn 118(ppb)	0.05	0.05	0.03	0.10	3.00	3.00	3.00
Cd 111(ppb)	0.01	0.01	0.01	0.02	0.70	0.70	0.70
Sb 121(ppb)	0.03	0.03	0.02	0.06	2.00	2.00	2.00
Ba 137(ppb)	2.89	0.42	0.25	0.76	25.00	25.00	25.00
Tl 205(ppb)	0.02	0.02	0.02	0.02	0.70	0.70	0.70
Pb 206(ppb)	0.10	0.10	0.06	0.18	6.00	6.00	10.42
Th 232(ppb)	0.01	0.01	0.01	0.01	0.40	0.40	0.40
U 238(ppb)	0.01	0.01	0.00	0.01	1.46	2.65	1.55
V 51(ppb)	0.35	0.11	25.24	230.73	6.30	6.30	6.30
Cr 52(ppb)	0.15	0.15	0.08	0.30	8.40	8.40	8.40
Fe 56(ppb)	5.53	5.53	3.30	10.00	337.00	337.00	337.00
As 75(ppb)	0.48	0.05	0.36	6.92	2.01	2.10	2.19

Tab. S2: PCB retrieved concentrations in Arctic and Antarctic lakes

a) Arctic

	Water					Sediment				
	Solvannet L1W	Glacier L2W	Knudsenheia L3W	Storvatnet L4W	Tvillingvatnet L5W	Solvannet L1S	Glacier L2S	Knudsenheia L3S	Storvatnet L4S	Tvillingvatnet L5S
∑ PCB31 + 28	0.08	0.08	0.09	0.07	0.07	0.11	0.09	0.09	0.09	0.10
PCB52	0.00	0.00	0.00	0.00	0.00	0.15	0.06	0.10	0.11	0.10
PCB74	0.06	0.00	0.00	0.00	0.00	0.14	0.00	0.03	0.03	0.02
∑ PCB84 + 90 + 101	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.04	0.04	0.04
PCB99	0.16	0.00	0.00	0.00	0.00	0.09	0.00	0.00	0.00	0.00
PCB81	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
PCB110	0.00	0.00	0.00	0.00	0.00	0.20	0.00	0.03	0.06	0.11
PCB77	0.00	0.00	0.00	0.00	0.00	0.06	0.00	0.00	0.00	0.00
PCB151	0.00	0.00	0.00	0.00	0.10	0.07	0.00	0.00	0.00	0.03
PCB149	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.03	0.00	0.06
PCB123	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00
PCB118	0.00	0.00	0.00	0.00	0.04	0.22	0.00	0.07	0.03	0.07
∑ PCB153 +168	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.05	0.03	0.05
PCB105	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00	0.00	0.00
∑ PCB138 +158	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.02	0.00	0.03

b) Antartic

	Water							Sediment						
	Argentina LAW	Sofia LSW	Crater LCW	Zapatilla LZW	Extremadura LEW	Telefon LTW	Ballaneros LBW	Argentina LAS	Sofia LSS	Crater LCS	Zapatilla LZS	Extremadura LES	Telefon LTS	Ballaneros LBS
∑ PCB31 + 28	0.10	0.09	0.11	0.09	0.10	0.11	0.10	0.09	0.09	0.07	0.08	0.09	0.09	0.09
PCB52	0.06	0.03	0.10	0.05	0.05	0.10	0.09	0.10	0.10	0.08	0.09	0.09	0.10	0.10
PCB74	0.05	0.04	0.09	0.04	0.09	0.06	0.06	0.10	0.10	0.08	0.09	0.04	0.10	0.06
∑ PCB84 + 90 + 101	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.09	0.10	0.08	0.08	0.09	0.09	0.09
PCB99	0.05	0.00	0.14	0.07	0.19	0.12	0.11	0.04	0.00	0.00	0.09	0.05	0.04	0.03
PCB81	0.07	0.05	0.08	0.06	0.11	0.09	0.12	0.00	0.00	0.07	0.04	0.04	0.00	0.09
PCB110	0.00	0.00	0.00	0.05	0.08	0.02	0.08	0.10	0.10	0.08	0.09	0.09	0.10	0.10
PCB77	0.02	0.00	0.06	0.00	0.05	0.07	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PCB151	0.03	0.00	0.14	0.00	0.19	0.12	0.14	0.03	0.03	0.06	0.00	0.00	0.03	0.05
PCB149	0.02	0.00	0.00	0.00	0.11	0.02	0.08	0.08	0.08	0.00	0.05	0.06	0.08	0.05
PCB123	0.03	0.00	0.00	0.10	0.09	0.11	0.11	0.06	0.09	0.00	0.04	0.05	0.06	0.03
PCB118	0.00	0.00	0.00	0.02	0.09	0.06	0.02	0.09	0.10	0.08	0.09	0.10	0.10	0.10
∑ PCB153 +168	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.08	0.06	0.05	0.06	0.07	0.07
PCB105	0.09	0.00	0.11	0.11	0.11	0.15	0.06	0.00	0.03	0.00	0.00	0.00	0.03	0.00
∑ PCB138 +158	0.00	0.03	0.00	0.00	0.05	0.00	0.09	0.00	0.07	0.00	0.00	0.00	0.07	0.06

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microorganisms

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Article

Enrichment, Isolation and Characterization of Heavy Metal-Tolerant Bacteria from Polar Lacustrine Sediments

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Abstract: Polar areas are not exempt from anthropogenic pollution. Heavy metals have been detected in Arctic and Antarctic lakes. Bacteria, at the base of the food web, can possess the ability to adsorb or immobilize heavy metals in the environment and reduce their concentration in the water column. However, several gaps exist in our knowledge of bacterial tolerance to heavy metals in polar systems, especially in lakes. Heavy metal-tolerant bacteria from polar lacustrine sediments were selectively enriched and subsequently isolated and identified. Their growth at increasing concentrations of different heavy metals (iron, copper, and mercury) was evaluated. Selected isolates were tested for sequestration of iron and mercury. A total of 101 bacterial isolates were obtained from metal-enriched cultures. Gammaproteobacteria and Actinomycetota isolates were most abundant in Arctic and Antarctic enrichments, respectively. Iron was the most tolerated metal. Mercury and iron were sequestered by the isolates by up to 14.2 and 13.4%, respectively. The results from this study contribute to our understanding of heavy metal-tolerant bacteria from cold environments and their potential use in biotechnological applications.

Keywords: heavy metal tolerance; *Pseudomonas*; lacustrine sediments; bacterial isolates; biosequestration

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1. Introduction

The widespread perception is that Polar Regions are pristine. Nevertheless, by a process of long-range transport (across national borders at varying rates, e.g., through pathways like water or air), they can be reached by a plethora of contaminants originating from thousands of miles away, thus becoming a sink and potential reservoir of legacy pollutants [1–3]. Lakes lying in extremely remote areas, such as the High Arctic and Antarctica, are sensitive reference systems for measuring human impacts such as global climate change and long-range pollution transport. Despite the adoption of precautions, they have become increasingly affected by airborne long-range and locally emitted (e.g., from research activities and tourism) organic and heavy metal contaminants [4,5]. In

the Arctic, military installations, industrial emissions, mining activities, small settlements, power generators, and vehicles are all local sources of pollution [6]. In aquatic environments, lake sediments act as a sink for metals, where they can accumulate at concentrations higher than those in the overlying waters [7]. Several investigators have demonstrated the occurrence of heavy metals in the sediments of Arctic [3,8–10] and Antarctic [11] lacustrine systems. Polar lakes are dominated by the microbial loop, a model that describes the cycling of carbon and nutrients through microorganisms, including viruses, bacteria, phytoplankton, zooplankton, and protozoa [12], in aquatic environments. Among these, bacteria are the most prevalent sedimentary organisms, representing the first step in the transfer of toxic compounds, including metals, to higher trophic levels. By means of their genetic and biochemical capabilities, bacteria can adopt strategies (via metabolic pathways or resistance mechanisms) allowing them to adsorb, accumulate, and transform heavy metals in most food chains [13], with implications for their repurposing in bioremediation. A number of metals are essential micronutrients for microbial growth (e.g., zinc, copper, nickel, chrome, iron) and are involved in cellular processes such as redox reactions, as coenzymes, and in osmotic regulation [14]. Conversely, some elements are nonessential (e.g., cadmium, mercury, and lead), toxic, and harmful for physiological functions in bacteria at all concentrations, including at sub-chronic doses [15]. According to Chandrangsu et al. [16], the genetic features of the microbiomes could be significantly modified due to the effects of elevated concentrations of heavy metals and/or other pollutants in the environment. Long-term exposure to high chemical concentrations may cause bacteria to become resistant by developing new defense mechanisms against the stress caused by harmful substances. Thus, the presence of metal-resistant bacteria can become bioindicators of pollution events due to their microbial adaptive strategies [17].

To date, our current knowledge of heavy metal-tolerant bacteria isolated from Arctic and Antarctic environments remains scarce and fragmentary. Studies have mainly addressed heavy metal tolerance in bacteria from soil [18–20], marine biota [21,22], marine environments [23–26], and riverine systems [27,28], whereas few investigations have focused on bacteria from polar lakes [29]. Therefore, there are still many gaps to fill in our knowledge of the interactions between microorganisms and elements in extremely cold environments, and especially lacustrine systems.

The goal of this study was to isolate bacteria with potential for bioremediation applications. This was achieved by (i) enriching and isolating microorganisms from lacustrine sediment with tolerance for heavy metals under cold conditions, (ii) identifying the bacterial isolates, and (iii) assessing their metal biosequestration capability.

2. Materials and Methods

2.1. Sampling Activities

Sampling activities were carried out in eleven shallow lakes lying in the Arctic (four lakes in Ny-Ålesund, Svalbard Islands, Arctic Norway; coordinates 78°55′ N–11°56′ E) and Antarctica (seven lakes between Deception Island, coordinates 62°34′35″ S–60°54′14″ W, and Livingston Island, coordinates 62°57′ S–60°38′ W, in the South Shetland Islands). In the Arctic, sampling was performed between August 5th and 18th 2021 from lakes surrounding the Ny-Ålesund research village, as follows: Lake Solvannet (L1), Lake Glacier (L2), Lake Knudsenheia (L3), and Lake Storvatnet (L4). In Antarctica, sampling was performed between 25 January and 1 February 2022 from the following lakes: Lake Argentina (LA) and Lake Sofia (LS) (both in Livingston Island), Lake Ballaneros (LB), Lake Crater (LC), Lake Extremadura (LE), Lake Telefon (LT), and Lake Zapatilla (LZ) (Deception Island) (Table 1). The lakes Solvannet, Storvatnet, Argentina, and Crater were selected for

sampling as impacted lakes because of their location (e.g., near the scientific bases) and their characteristics (e.g., presence of animals).

Table 1. Arctic and Antarctic lakes investigated in this study. The main features of the sampled lakes are indicated in the last column: A/A = lake influenced by animals and humans; B = brackish lake; G = glacial lake.

Polar Area	Lake	Lake ID	Coordinates	Physical–Chemical Parameters				
				Temp. (°C)	pH	O_2 (%)	Cond. (uS/cm)	Main Features
Ny-Ålesund (Arctic Norway)	Solvannet	L1	78°55.552′ N–11°56.327′ E	6.2	8.17	98.0	395	A/A
	Glacier	L2	78°55.044′ N–11°47.442′ E	8.7	7.66	98.2	151	G
	Knudsenheia	L3	78°56.680′ N–11°51.579′ E	8.8	8.36	105.3	2620	B
	Storvatnet	L4	78°55.453′ N–11°52.728′ E	7.9	8.07	103.2	243	A/A
Livingston Island (Antarctica)	Sofia	LS-1	62°40′12.19″ S–60°23′17.90″ W	0.3	5.2	69.4	26.5	G
	Argentina	LA	62°40′22.39″ S–60°24′18.12″ W	1.4	5.7	68.1	64.6	A/A
Deception Island (Antarctica)	Crater	LC	62°59′00.68″ S–60°40′20.41″ W	3.7	5.5	86.0	6.64	A/A
	Zapatilla	LZ	62°59′00.24″ S–60°40′29.07″ W	6.8	5.6	76.5	55.6	Water supply
	Extremadura	LE	62°55′12.2″ S–60°39′47.0″ W	4.1	7.2	94.8	448	B
	Telefon	LT	62°55′39.9″ S–60°41′21.3″ W	5.4	6	-	507	B
	Ballaneros	LB	62°58′51.1″ S–60°34′27.1″ W	4.1	4.7	67.9	480	B

Trace Fe concentrations in the analyzed lakes ranged between 3.3 and 10 μg L^{-1}, except for brackish lakes, where concentrations were approximately 300 μg L^{-1}. A similar situation was found for Cu, which ranged from 0.5 to 3.9 μg L^{-1}, except in brackish lakes, where values ranged between 291 and 601 μg L^{-1}. Hg concentrations ranged between 0.2 and 1 μg L^{-1}.

At each lake, water and sediment samples (depth 30–60 cm) were aseptically collected. Water (6 L) was collected in pre-sterilized polycarbonate containers. Sediments (first 10 cm; 200 g) were collected using a pre-sterilized metal scoop and sterile plastic containers. Samples were maintained at 4 °C during transportation to the laboratories of the Research Bases for preliminary processing. The main physical–chemical parameters (O_2, pH, temperature, and conductivity) were recorded.

2.2. Setup of Enrichment Cultures

Enrichment cultures were set up by using 1 g of wet lake sediment to inoculate 75 mL of filter-sterilized lake water. Cultures were enriched with individual heavy metal (HM) salts, as follows: $FeSO_4$ (Fe), $CuSO_4$ (Cu), and $HgCl_2$ (Hg) (Sigma, Burlington, MA, USA). Metal salt solutions were prepared in filter-sterilized 1X phosphate-buffered saline (PBS), as reported by Selvin et al. [30]. Fe and Cu were added at a final concentration of 1000 mg L^{-1}, whereas Hg was added at a final concentration of 100 mg L^{-1}. All enrichments were incubated aerobically at 4 °C for two months [27,28].

2.3. Isolation of HM-Tolerant Bacteria

Aliquots (100 μL) of each enrichment culture were spread-plated in duplicate on HM-amended nutrient agar (NA; Difco, Milan, Italy) using the same HM concentrations as in the enrichment setup (i.e., 1000 mg L^{-1} for Fe and Cu and 100 mg L^{-1} for Hg). Inoculated plates were incubated at 4 °C for 30 days. Bacterial colonies grown on HM-amended agar plates were randomly selected, picked, and subcultured on NA under the same conditions. HM-tolerant bacterial isolates were routinely grown on NA and maintained at 4 °C.

2.4. Bacterial Growth at Increasing HM Concentrations

A microtiter plate-based culture assay was set up to screen bacterial isolates for growth at increasing concentrations (up to 5000 mg L^{-1} for Fe and Cu, and up to 500 mg L^{-1} for Hg) of the same HM by amending the enrichment of the original culture. A modified version of the protocol described by Blasi et al. [31] was applied. Briefly, starting bacterial cultures were prepared by growing each bacterial strain in a microtiter plate well, as follows: Each well was filled with 250 µL of nutrient broth (NB) in duplicate. The plates were then incubated at 4 °C for 15 days. Bacterial growth was assessed by measuring the optical density of the cultures over time at 605 nm (OD605), using a microplate reader (Adsorbance 96 spectrophotometer, Byonoy, Hamburg, Germany). The recorded values were processed using the Absorbance 96 software version 1.2. Wells containing NB without bacterial inoculation were used as blanks for the OD readings. After incubation, 25 µL of each strain culture were transferred, in duplicate, into 225 µL of 10% NB amended with the HMs at the following concentrations: 2500 and 5000 mg L^{-1} for Fe and Cu, and 250 and 500 mg L^{-1} for Hg. The plates were then incubated at 4 °C for 15 days. Growth was assessed daily as described above. Wells containing 10% HM-amended NB (without bacterial inoculation) were used as negative controls.

2.5. Screening for Multitolerance

Bacterial strains capable of growing in the presence of high concentrations of HMs were further assayed for tolerance to HMs different from those used in the enrichment cultures. Selected strains were streaked on NA amended with HMs and incubated for 30 days at 4 °C. The concentrations used were 1000, 2500, and 5000 mg L^{-1} for Fe and Cu, and 100 mg L^{-1} and 250 mg L^{-1} for Hg [28].

2.6. Sequestration Rate of Heavy Metals

To test the HM sequestration rate, selected bacterial strains were inoculated in NB amended with 190 mg L^{-1} of mercury or 2250 mg L^{-1} of iron. To monitor abiotic losses, two negative controls were prepared and treated as the inoculated cultures: NB (i.e., without bacterial inoculation or metal addition) and NB*plus*HM (i.e., without bacterial inoculation but with the addition of 190 mg L^{-1} for Hg or 2250 mg L^{-1} for iron). After incubation for 30 days at 4 °C, the samples were centrifuged at 8000 rpm for 20 min at 4 °C to separate the liquid phase from the pellet. The samples for analysis were diluted (1:1000) in a solution of one liter containing 0.1% of Triton X, 1% of nitric acid, and 0.1% of EDTA, and then analyzed by Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES; characteristics of the instrument in [32]), according to Turetta et al. [33].

2.7. Identification of Bacterial Isolates

Bacterial isolates were identified by 16S rRNA gene sequencing. Briefly, single bacterial colonies were lysed by heating (95 °C for 10 min). Amplification of the 16S rRNA gene was performed with a thermocycler (Mastercycler GeneAmp PCR-System 9700, Applied Biosystems, Waltham, MA, USA) using the bacteria-specific primers 27F (5′–AGAGTTTGATC(AC)TGGCTCAG–3′) and 1492R (5′–TACGGYTACCTTGTTACGAC-3′). The reaction mixtures were assembled at 0 °C. The composition of the reaction mixture was (final volume: 25 µL) 2 µL DNA, 1 µL of each of the two primers (10 µM), 5 µL of reaction buffer 10×, 0.25 µL of Taq polymerase My Taq PRIME (5 U μL^{-1}), and sterile Milli-Q water. Negative controls for DNA extraction and PCR setup (reaction mixture without a DNA template) were also used in every PCR run. The PCR program used was (1) 95 °C for 1 min; (2) 35 cycles at 95 °C for 30 s, 52 °C for 30 s, and 72 °C for 30 s; and (3) 72 °C for 10 min. Quality of the DNA was checked by running 2 µL of each sam-

ple on 1% agarose gel (*w*/*v*) (Bioline, Meridian Bioscience, Menphis, TN, USA) in TAE buffer 1X (0.04 M Tris-acetate, 0.02 M acetic acid, 0.001 M EDTA) (Fermentas, Waltham, MA, USA) containing SYBR™ Safe (1 µL/25mL final concentration) (Invitrogen, Waltham, MA, USA). Amplified products were sequenced by Eurofins Europe (Konstanz, Germany). The FinchTV (version 1.4) program was used to read and correct the manually obtained sequences. Next relatives of isolates were determined by comparing sequences in the NCBI GenBank and the EMBL databases using BLAST and the "Seqmatch" and "Classifier" programs of the Ribosomal Database Project II (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&BLAST_SPEC=GeoBlast&PAGE_TYPE=BlastSearch, accessed on 5 February 2025) [34]. Sequences were further aligned using the program Clustal W (webpage link https://www.genome.jp/tools-bin/clustalw, accessed on 5 February 2025) to the most similar orthologous sequences retrieved from databases.

2.8. Statistical Analysis

Results obtained for microbial growth in the enrichment cultures and metal concentrations in the analyzed samples were used to perform linear regression and Spearman correlation, using R software version 4.4.2 with the base R package stats version 4.4.2.

3. Results

3.1. Bacterial Isolation

Overall, 58 and 43 bacterial strains were isolated from Arctic and Antarctic lakes, respectively. In particular, 90 were from Fe enrichments (1000 mg L^{-1}); 50 and 40 were from Arctic and Antarctic lakes, respectively, i.e., 16 from Lake Knudsenheia, 13 from Lakes Solvannet, 11 from Lake Glacier, and 10 from Lake Storvatnet in the Arctic, and 13 from Lake Telefon, 13 from Lake Ballaneros, 7 from Lake Zapatilla, 4 from Lake Extremadura, and 3 from Lake Argentina in Antarctica. Additionally, eight were from the Hg enrichments (100 mg L^{-1}), all from the Arctic Lake Storvatnet, and three were from Cu enrichments (1000 mg L^{-1}), all from the Antarctic Lake Ballaneros.

3.2. Bacterial Growth at Increasing HM Concentrations

As stated above, all isolates were then tested for growth at metal concentrations higher than those used for the enrichments, i.e., Fe and Cu up to 5000 mg L^{-1} and Hg up to 500 mg L^{-1}. None of the Cu- and Hg-tolerant strains were able to grow at higher concentrations of Cu and Hg. Conversely, 10 and 15 isolates from Antarctic and Arctic lake water, respectively, enriched with Fe, were able to grow in the presence of up to 5000 mg L^{-1} of Fe, even if, in some cases, bacterial growth was weak (Table 2).

Fe-tolerant bacterial isolates were successfully identified by 16S rRNA gene sequencing and their phylogenetic affiliation is shown in Supplementary Table S1. Sequences of six isolates were not obtained due to poor amplification and/or bad sequencing quality.

The majority of identified Fe-tolerant bacterial isolates from Arctic lake sediments were affiliated with the genus *Pseudomonas* (within Gammaproteobacteria). Additionally, two strains belonged to the genus *Janthinobacterium* (within Betaproteobacteria), while a single strain was affiliated with the genus *Carnobacterium* (within Bacillota). Unfortunately, identification was not successful for four strains (i.e., S2A-1, S2A-4, S2A-5, and S4A-2).

Identified Fe-tolerant bacterial isolates from Antarctic lake sediment were mainly affiliated with the phylum Actinomycetota, within the genera *Subtercola*, *Pseudoarthrobacter,* and *Arthrobacter*. Two other isolates were affiliated with the genus *Janthinobacterium* (within Betaproteobacteria). Unfortunately, the phylogenetic affiliation could not be determined for two strains (ABA-4 and AZA-3). Antarctic isolates are part of the Italian Collection of Antarctic Bacteria of the National Antarctic Museum (CIBAN-MNA), kept

at the University of Messina (voucher codes MNA-CIBAN-2266 to MNA-CIBAN-2275; Supplementary Table S1).

Table 2. Arctic and Antarctic isolates growing at increasing concentrations of Fe. (+), weak growth; +, normal growth; ++, conspicuous growth.

Lake of Origin	Isolate	Fe (mg L^{-1})	
		2500	5000
Arctic Lakes			
Lake Solvannet (L1)	*Pseudomonas* sp. S1A-14	+	+
Lake Glacier (L2)	S2A-1 (not identified)	+	+
	S2A-2 (not identified)	+	+
	Carnobacterium sp. S2A-4	+	(+)
	S2A-5 (not identified)	+	(+)
	Janthinobacterium sp. S2A-6	(+)	(+)
	Janthinobacterium sp. S2A-7	+	+
	Pseudomonas sp. S2A-8	++	+
Lake Knudsenheia (L3)	*Pseudomonas* sp. S3A-2	+	+
	Pseudomonas sp. S3A-11	+	+
Lake Storvatnet (L4)	*Pseudomonas* sp. S4A-1	(+)	(+)
	S4A-2 (not identified)	+	+
	Pseudomonas sp. S4A-4	+	+
	Pseudomonas sp. S4A-7	+	(+)
	Pseudomonas sp. S4A-10	+	+
Antarctic Lakes			
Lake Ballaneros (LB)	ABA-4 (not identified)	(+)	(+)
	Arthrobacter sp. ABA-13	(+)	(+)
Lake Argentina (LA)	*Pseudarthrobacter* sp. AAA-2	++	+
	Pseudarthrobacter sp. AAA-4	+	(+)
Lake Zapatilla (LZ)	*Pseudarthrobacter* sp. AZA-2	++	+
	AZA-3 (not identified)	++	(+)
	Pseudarthrobacter sp. AZA-4	++	+
	Subtercola sp. AZA-8	++	(+)
	Subtercola sp. AZA-9	+	(+)
Lake Telefon (LT)	*Arthrobacter* sp. ATA-13	(+)	(+)

3.3. Screening for Multitolerance

Only four bacterial isolates from those that tolerated up to 5000 mg L^{-1} of Fe grew (even if weakly) at higher concentrations of Cu (Table 3).

Table 3. Arctic and Antarctic isolates tolerating more than one heavy metal salt. (+), weak growth; +, normal growth; −, no growth.

Polar Area	ID Strain	Cu (mg L^{-1})			Hg (mg L^{-1})		
		1000	2500	5000	100	250	500
Arctic	*Pseudomonas* sp. S1A-14	−	−	−	+	+	(+)
	S2A-1 (not identified)	−	−	−	−	−	−
	S2A-2 (not identified)	−	−	−	−	−	−
	Carnobacterium sp. S2A-4	−	−	−	−	−	−
	S2A-5 (not identified)	−	−	−	+	(+)	(+)
	Janthinobacterium sp. S2A-6	−	−	−	−	−	−
	Janthinobacterium sp. S2A-7	−	−	−	+	+	−
	Pseudomonas sp. S2A-8	(+)	−	−	+	(+)	−
	Pseudomonas sp. S3A-2	−	−	−	+	(+)	−
	Pseudomonas sp. S3A-11	(+)	−	−	+	+	−
	Pseudomonas sp. S4A-1	−	−	−	+	+	−
	S4A-2 (not identified)	−	−	−	+	+	−
	Pseudomonas sp. S4A-4	−	−	−	+	+	−
	Pseudomonas sp. S4A-7	−	−	−	+	+	−
	Pseudomonas sp. S4A-10	−	−	−	+	+	−

Table 3. *Cont.*

Polar Area	ID Strain	Cu (mg L^{-1})			Hg (mg L^{-1})		
		1000	2500	5000	100	250	500
Antarctica	ABA-4 (not identified)	(+)	(+)	(+)	(+)	(+)	–
	Arthrobacter sp. ABA-13	–	–	–	+	+	–
	Pseudarthrobacter sp. AAA-2	–	–	–	+	+	–
	Pseudarthrobacter sp. AAA-4	–	–	–	+	(+)	–
	Pseudarthrobacter sp. AZA-2	–	–	–	+	(+)	–
	AZA-3 (not identified)	–	–	–	+	(+)	–
	Pseudarthrobacter sp. AZA-4	–	–	–	+	(+)	–
	Subtercola sp. AZA-8	(+)	(+)	–	(+)	(+)	(+)
	Subtercola sp. AZA-9	–	–	–	–	–	–
	Arthrobacter sp. ATA-13	–	–	–	–	–	–

In detail, *Pseudomonas* spp. S2A-8 and S3A-11, from the Arctic, tolerated up to 1000 mg L^{-1} of this metal. Among the Antarctic isolates, the strain ABA-4 (not identified) tolerated up to 5000 mg L^{-1} of Cu, while *Subtercola* sp. AZA-8 grew at a Cu concentration of 2500 mg L^{-1}. A total of 16 bacterial strains (out of 25) tolerated Hg up to 250 mg L^{-1}, with better growth generally shown by Antarctic rather than Arctic isolates. Finally, only three strains tolerated Hg up to 500 mg L^{-1}; these were *Pseudomonas* sp. S1A-14 and S2A-5 (not identified) from Arctic lakes, and *Subtercola* sp. AZA-8 from Antarctica. Six isolates did not tolerate Cu and Hg at the tested concentrations.

3.4. Statistical Results

The statistical analyses carried out on obtained data did not show significant results, except for the Spearman table showing significative positive correlations among the number of colonies counted on Fe enrichment cultures and metal concentrations in the analyzed lakes (Supplementary Figure S1). Fe, Cu, and Hg were also positively correlated.

3.5. Sequestration Rate of Heavy Metals

Based on the results reported above, three and six isolates from Antarctica and the Arctic, respectively, were selected to carry out further evaluation of their sequestration rates for Fe and Hg. Even if some strains tolerated Cu, their growth in the presence of this metal was too weak to proceed with further tests. Isolates from the Arctic were *Pseudomonas* spp. S1A-14, S2A-8, and S3A-11; those not identified included S2A-1, S2A-5, and S4A-2. Antarctic isolates were *Pseudarthrobacter* spp. AAA-2 and AAA-4 and *Arthrobacter* sp. ABA-13. As shown in Figure 1a, both Arctic and Antarctic isolates exhibit a sequestration rate of Hg ranging from below 6% to 14.2%. No abiotic losses were observed in the control samples. The Arctic strain S2A-5 was the most promising isolate as it sequestered approximately 14.2% of the metal, followed by *Pseudomonas* spp. S1A-14 and S2A-8 (11.7 and 10.3%, respectively, both from Arctic lakes). For Fe sequestration (Figure 1b), the most promising strain was *Arthrobacter* sp. ABA-13 (13.4%) from Antarctica, followed by the Arctic *Pseudomonas* sp. S2A-8 (11.9%). The remaining bacterial isolate strains showed sequestration rates below 4%.

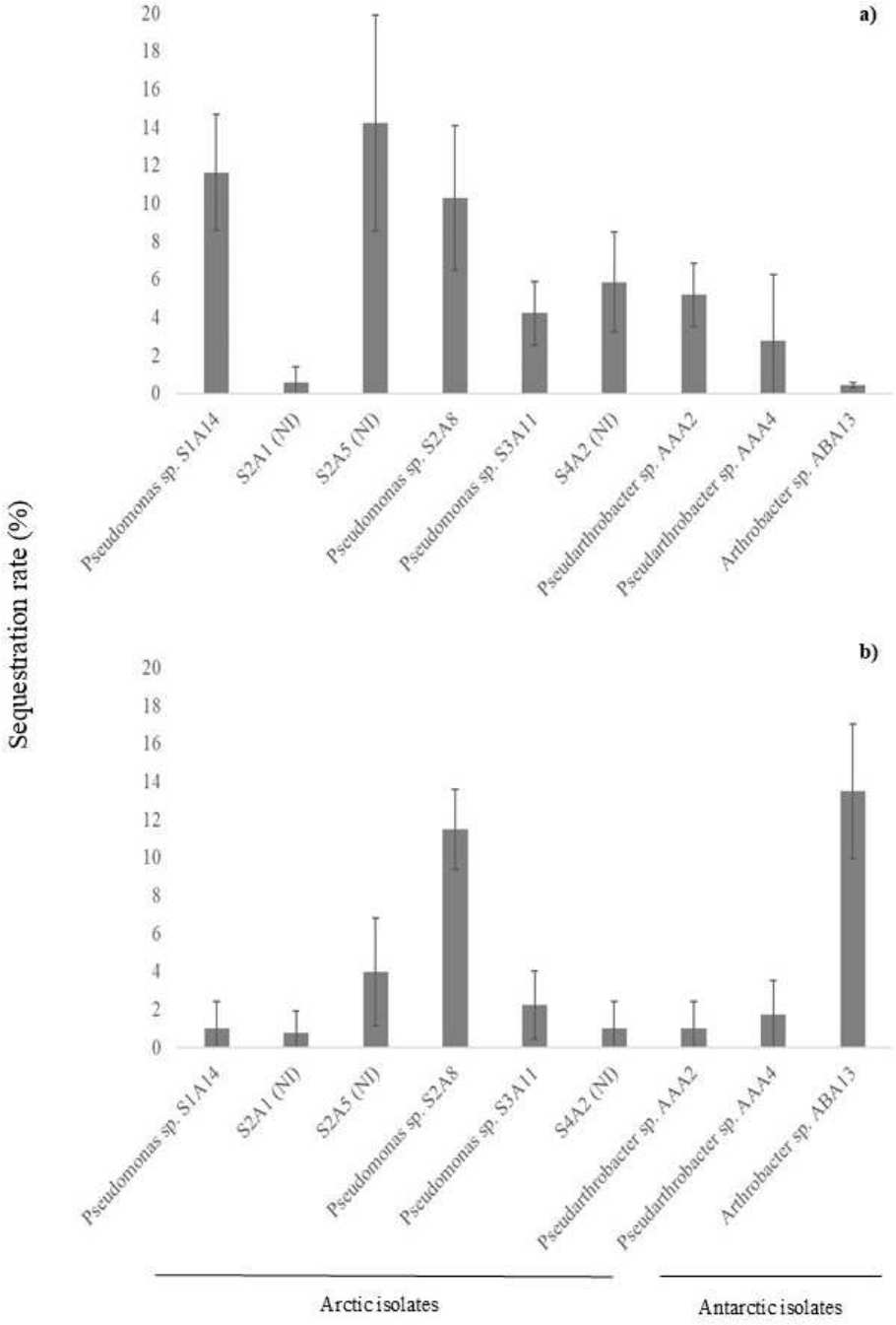

Figure 1. Sequestration of (**a**) mercury and (**b**) iron by Arctic and Antarctic bacteria.

4. Discussion

There is increasing interest in studying heavy metal tolerant-microorganisms from natural environments because of their high potential for industrial applications and their applicability in situ in bioremediation. To date, there is a lack of knowledge on the existence of heavy metal-tolerant cold-adapted bacteria (both psychrophilic and psychrotolerant), especially in lacustrine habitats in the Arctic and Antarctica. In this study, 58 heavy metal-tolerant bacterial isolates (to copper, iron, and mercury) were tested at 4 °C. Tests at higher concentrations of these metals showed that Fe was better tolerated than Cu and Hg. This finding is in line with previous studies reporting Fe resistance as a common trait in environmental bacteria, even in cold regions, likely due to its essential role in microbial metabolism and availability in sediment environments [14,35]. In fact, iron is crucial for

bacterial growth and survival, as it is a common co-factor in essential enzymes [36]. Natural lithogenic/geogenic sources of heavy metals resulting in naturally induced resistance should also be considered [37]. For instance, the profiles of the microbial resistome (genes related to antibiotic, biocide, and heavy metal resistances) were evaluated using a metagenomics approach in Antarctic areas with and without any anthropogenic impact [38]. While all resistance genes were found in impacted sites, only genes for heavy metal tolerance were retrieved from non-impacted regions.

To the best of our knowledge, the heavy metal-tolerant isolates in this study were closely related to taxa that have never been reported in the scientific literature as metal-resistant or -tolerant. On the contrary, some of them have been listed in two papers in relation to bacterial diversity in snow in Ny-Ålesund and antibiotic resistance [39,40]. While their presence in microbial diversity studies is expected, their antibiotic resistance capacity indicates an ability to adapt to environmental stresses. Given the well-known relationship between metal and antibiotic resistance, this reinforces the relevance of our study in characterizing these microorganisms in metal-rich environments.

A distinctive feature of the lake sediment we analyzed was the prevalence of Proteobacteria (mainly Gammaproteobacteria in the genus *Pseudomonas*) and Actinomycetota (genera *Arthrobacter*, *Pseudoarthrobacter,* and *Subtercola*) in the Arctic and Antarctic Fe-tolerant bacterial isolates, respectively. Actinomycetota could possess a slight advantage in iron sequestration due to their robust cell wall structures, which can facilitate enhanced metal binding. Conversely, Gammaproteobacteria may prioritize resistance mechanisms such as efflux systems over sequestration [41]. The genus *Janthinobacterium* (among Betaproteobacteria) was common to both regions, whereas a single *Carnobacterium* representative (among Firmicutes) was isolated from an Arctic lake. *Pseudomonas* and *Janthinobacterium* were among the prevalent genera harboring resistome genes in a study carried out in marine sediments at Deception Island [26]. *Pseudomonas* species show extraordinary resilience, thus surviving and becoming dominant in environments characterized by extreme conditions and limited nutrient availability. *Pseudomonas* members are known for their ability to reduce a wide variety of metals [36,37,42–44]. The resistome profile of an Antarctic *Pseudomonas* against multiple antibiotics, biocides and metals was analyzed recently [45]. *Janthinobacterium* was reported to not tolerate heavy metals, such as Ag, Cu, Hg, Pb, and Ni [46]. In a study by Yin et al. [47], the relative abundance of *Janthinobacterium* decreased in highly contaminated samples. The tolerance of *Carnobacterium* members to heavy metals, such as Cd and Sb, has been assessed [48], also in cold environments [44]. Finally, Actinomycetota are well known for their metabolic versatility and bioactive properties, which make them particularly attractive for application in bioremediation fields [49]. Heavy metal tolerance in cold-adapted *Arthrobacter* members has been frequently reported (e.g., [19,39,50,51]). Comprehensive genome sequencing of the psychrotolerant *Arthrobacter* sp. PAMC25284 from Antarctic revealed the presence of genes involved in copper resistance [52]. *Pseudoarthrobacter* members, from warmer areas, have seldom been reported as heavy metal-tolerant [53,54]. Finally, to the best of our knowledge, we report for the first time the ability of the genus *Subtercola* to grow in the presence of heavy metals, as its ability to grow even weakly at higher concentrations is remarkable. In this study, only the Arctic *Pseudomonas* sp. S1A-14 and S2A-5 (not identified) and the Antarctic *Subtercola* sp. AZA-8 were able to grow at 500 mg L^{-1} of Hg. In general, mercury was the least-tolerated metal, with most Arctic and Antarctic isolates growing only up to 250 mg L^{-1} of Hg.

Overall, this assessment of metal sequestration revealed limited capacities in the bacterial isolates tested. With respect to iron, the Antarctic *Arthrobacter* sp. ABA-13 and the Arctic *Pseudomonas* sp. S2A-8 achieved the highest rates, at about 13%. Similarly, 10–14% of Hg was removed from the culture medium, with an unidentified strain of S2A5

together with *Pseudomonas* sp. S2A-8 and *Pseudomonas* sp. S1A-14 (all from Arctic lakes) as the most promising isolates. These findings suggest that even if polar bacteria can tolerate heavy metals, their sequestration efficiency remains modest, potentially limiting their direct application in bioremediation [24]. Nevertheless, the genetic determinants of resistance in these taxa could provide valuable insights when developing biotechnological applications for heavy metal bioremediation in cold areas. Future work should focus on characterizing resistance genes and their regulatory pathways and exploring co-culture strategies to enhance sequestration rates. Therefore, observations from this study constitute a baseline for further analysis aimed at better elucidating the metabolizing capabilities of bacteria from polar lacustrine sediments.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microorganisms13020389/s1: Table S1: 16S rRNA gene sequence affiliation to their closest phylogenetic neighbors of Arctic and Antarctic isolates. Figure S1: Spearman correlation table for Fe, Cu, and Hg concentrations in all analyzed lakes, along with the number of bacterial colonies grown in metal-enriched cultures. The table includes correlation coefficients and *p*-values, while the visualization highlights statistically significant correlations ($p < 0.05$) using ellipses, with numbers indicating the strength and direction of the correlation.

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PCB-Oxidizing Bacteria in Arctic and Antarctic Lakes: Insights into Bioremediation Potential in Polar Ecosystems

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Abstract

Polychlorinated biphenyls (PCBs) are long-lasting organic pollutants commonly found in polar regions due to their ability to be transported and deposited over long distances in the atmosphere. This study examined the levels of PCB contamination in water and sediment samples taken from Arctic lakes (Ny-Ålesund, Svalbard) and Antarctic lakes (Livingston Island and Deception Island) and evaluated the biodegradation capabilities of local cold-adapted bacteria. The analysis revealed that Antarctic lakes had higher PCB concentrations compared to their Arctic counterparts, with sediments acting as significant reservoirs for these pollutants. Variability was noted among different lakes, with Deception Island exhibiting higher PCB levels, likely due to local environmental and volcanic influences. Several bacterial strains that were able to grow with PCBs as sole carbon and energy sources, were isolated and identified from sediment samples, mainly belonging to the genus Pseudomonas. Arctic isolates showed greater biodegradation efficiency, particularly those with the *bph*A gene, which is at the base for PCB degradation. These results underscore the essential role of polar lakes as both reservoirs and key areas for PCB degradation, while also highlighting the potential of cold-adapted bacteria for bioremediation in extreme environments.

Keywords

Cold-adapted bacteria, Polychlorobiphenyls, Contamination level, Arctic Lakes, Antarctic Lakes, Biodegradation.

Introduction

Arctic and Antarctic regions are characterized by a vast network of lakes and ponds that play a critical role in the overall biodiversity and biogeochemical cycling of these remote ecosystems (Rautio et al., 2011). Polar lakes often exhibit unique physical and chemical properties, such as permanent ice-cover, prominent haloclines, and low temperatures, which shape the composition and function of their

resident microbial communities (Li & Morgan-Kiss, 2019). While some studies have explored the general microbial diversity of polar lakes (Ntougias et al., 2016, Xing et al., 2020), a limited understanding of the specific potential for bioremediation of persistent organic pollutants (POPs), which can accumulate and persist even in these environments, still remains (Aoi et al., 2019). Among POPs, polychlorinated biphenyls (PCBs), due to their distinctive chemical and physical properties, were manufactured and utilized extensively in various industrial applications from 1929 until the late 1970s (Kodavanti, 2014). Despite the phaseout or restriction of these chemicals, they persist in the environment at levels that can negatively impact human and animal health because of their bioaccumulation and biomagnification properties (Cecinato et al., 2000, Jiao et al., 2009, Wang et al., 2009, Lehtinen et al., 2013). The issue of the transport and fate of PCBs in remote areas, such as the Arctic and Antarctica, has received increasing attention during the past decades (Carlsson et al., 2018, Aslam et al., 2019, Kovshov, 2019, Giannarelli et al., 2019). Lakes are mainly subjected to the accumulation of organic pollutants due to atmospheric deposition, becoming sinks for compounds transported from other regions (Carlsson et al., 2018, Giannarelli et al., 2019). Organic pollutants can be transported to glaciers and high-altitude environments by volatilization and condensation processes, known as the "cold condensation" (Oehme, 1991, Carlsson et al., 2018 Vecchiato et al., 2015a). It means that they can be deposited during the winter with the snow deposition and then released into the environment during the melting season (Grigoryeva et al., 2019, Vecchiato et al., 2015b). Considering the prevalence of PCBs in polar lacustrine environments (Bettinetti et al., 2008, Carlsson et al., 2018), understanding the potential for bioremediation through the study of PCB-oxidizing bacteria is crucial to disentangle their role in mitigating the environmental impact of these POPs. Once introduced into the environment, the fate of PCBs is intrinsically connected to bacteria, which serve as the initial step in the transfer of these toxic compounds to higher trophic levels (Gillan et al., 2005, Lo Giudice et al., 2013). Bacteria possess genetic and biochemical capacities for the remediation of PCB pollution. The biodegradation of specific, typically lower-chlorinated PCB congeners has been documented for microorganisms in the Arctic and Antarctic regions (Mohn et al.,

1997, Master and Mohn, 1998, Yakimov et al., 1999, Michaud et al., 2007, Lo Giudice et al., 2010, 2013). However, cold-adapted PCB-oxidizers have been rarely isolated from lake ecosystems, particularly in the Arctic and Antarctica (Li et al., 2009, Rappazzo et al., 2019, Papale et al., 2022).

In this context, the present study was aimed at quantifying PCBs in polar lacustrine systems and assessing the potential of autochthonous bacteria for PCB degradation. We focused on i) isolating bacterial strains capable of utilizing PCBs as their sole carbon and energy source, ii) identifying these strains through molecular techniques, iii) and evaluating their degradation efficiency at 4° C under controlled conditions.

Materials and Methods

Sample collection

Arctic samples were collected in the area of Ny-Ålesund (78°55' N, 11°56' E), Svalbard, High Arctic Norway, between 5 and 18 August 2021 from five different lakes, including Solvannet (L1), Glacier (L2), Knudsenheia (L3), Storvatnet (L4) and Tvillingvatnet (L5) (fig. 1a). Antarctic samples were collected on two islands in the Antarctic Peninsula, namely Livingston Island (62°34’35” S - 60°54’14” W) and Deception Island (62°57'S, 60°38'W), between 25 January and 1 February 2022 from 7 lakes, namely Argentina (LA) and Sofia (LS) (both in Livingston Island), Ballaneros (LB), Crater (LC), Extremadura (LE), Telefon (LT) and Zapatilla (LZ) (all in Deception Island) (fig. 1b). Geographical coordinates and physical-chemical data of water for each sampling point are shown in table 1. For each lake, water and sediment samples were collected from three almost equidistant points, except for the Antarctic lakes Crater (LC), Extremadura (LE) and Zapatilla (LZ) from which one or two sampling points were considered due to difficult sampling conditions (table 1). All samples were aseptically collected and preliminary processed after sampling (approximately 2 h) in the laboratory of the Italian Station Dirigible Italia in Ny-Ålesund (Arctic campaign) and in the

laboratories of Spanish stations Gabrielle de Castilla and Juan Carols I (Antarctic campaign), as described in the following sections.

PCB concentration in natural samples

Water samples were collected in triplicate using 100-mL glass vials. Sediment samples were collected using a pre-cleaned stainless steel bailer and stored in pre-cleaned glass jars until analysis. Chemical analyses to determine the PCB concentration in water and sediments samples were carried out by GC–MS/MS. The analytical procedure for PCB determination in water samples has been described elsewhere (Papale et al., 2024). The method consisted of a high efficiency micro extraction by MEPS (Micro Extraction by Packed Sorbent) followed by a gas chromatographic analysis with a mass spectrometric confirmation.

After collection, water samples (about 15 mL each) were immediately subjected to extraction. The extraction step allowed obtaining about 1000-fold concentration enrichment.

MEPS consists, in our case, of a micro-column packed with a C18 sorbent phase, inserted into a needle of a syringe referred as B.I.N. (Barrell Insert and Needle). The sorbent phase is highly non-polar and capable of retaining non-polar organic analytes and removing them from the aqueous matrix. The procedure allows for working with smaller volumes of sample and solvents compared to SPE, concentrating the target analytes more effectively, and removing interfering molecules.

A 500 µl MEPS syringe was conditioned with solvents progressively more similar to the matrix being analyzed. In the case of lake water, a polar matrix, the conditioning sequence was as follows: 500 µl of a hexane:ethyl acetate 1:1 solution, 500 µl of methanol (CH_3OH), and 500 µl of water. After conditioning the cartridge, cycles of aspiration and ejection are performed on a 15 ml sample aliquot. After ejection, the sample volume was discarded. Once the water sample was exhausted, the 500 µl

121 syringe was replaced with a 50 µl syringe, and elution was performed with 25 µl of isooctane into a
122 vial analysis. At the end of the procedure, the BIN was washed with isooctane.

123 Ultrasonication was the technique used for the removal of analytes from the sediments and their
124 transfer into the organic solvent.

125 Hexane:Acetone 1:1 was used as the solvent, as hexane is non-polar and allows the analytes to be
126 retained once removed from the solid matrix, while acetone helps to transport hexane (to which it is
127 compatible) through any residual water, preventing the formation of a biphasic system due to the
128 immiscibility of hexane in water.

129 A Solid Phase Extraction (SPE) technique was used for the purification of the analytes extracted from
130 the sediments.

131 Sediment samples were powdered using mortar grinding, with a 3 g aliquot placed into a 50 mL vial
132 fitted with an aluminum crimp cap. Then, 10 mL of hexane:acetone 1:1 solution and 10 µL of method
133 standard were added. The mixture was sonicated for 15 minutes at room temperature. Afterward, the
134 samples were centrifuged, and the supernatant was collected using a Pasteur pipette. The ultrasound
135 and centrifugation cycle was repeated two more times, each time adding 5 mL of solvent per cycle.
136 Once the supernatant was collected, it was reduced to 2 mL using a centrifugal evaporator. The
137 solvent was then changed by adding 2 mL of isooctane and reducing the volume to 1 mL.

138 To each sample 10 µl of an injection standard (MBP-CP solution/mixture of PCB77, PCB81, PCB126
139 and PCB169, conc. 10 µg ml− 1; Wellington Laboratories) was added before being injected into the
140 gas chromatograph.

141 The instrument used was an Agilent GC 7890B – MS 7010 (QQQ), the installed column was a HP-
142 5MS UI capillary column (5 % phenyl-methyl-polysiloxane), 30 meters in length, with an internal
143 diameter of 0.25 mm and a stationary phase thickness of 0.25 µm.

Before the sample injection, the instrument was calibrated with solutions of varying concentrations.

The analysis results were processed using Mass Hunter QQQ Quantitative Analysis® software. The automatic integration of the quantitative analysis curves was verified, with possible manual revision based on comparison with the corresponding qualitative analysis.

Helium 99.995% purity (Rivoira, Italy) was used as a carrier gas at constant flow (1,2 ml min^{-1}) and 90 kPa at initial temperature. The sample volume injected was 1 µl, unless otherwise specified.

For the instrumental analyses the limits of detection (LOD) and quantification (LOQ) were calculated. For the LOD, a value three times the standard deviation of the lowest concentration standard solution used for instrument calibration was considered, while for the LOQ, a value ten times the standard deviation was used. These limits were expressed as concentrations and converted according to the calibration curves for each individual analyte.

$$LOD_x = (3 * D.Std_x - Intercept_x) / Slope_x$$

$$LOQ_x = (10 * D.Std_x - Intercept_x) / Slope_x$$

where *x* represents the analyte for which the instrumental limit is being calculated and for which calibration was previously performed, D.Std. is the standard deviation of the result detected by the instrument for the lower concentration calibration solution, and the intercept and slope are parameters of the calibration curve for analyte *x*.

Set-up of bacterial cultures

Based on chemical results, only sediment samples were used for the set-up of enrichment cultures. or the enrichment cultures, biphenyl (BP) was used as the sole carbon and energy source to support microbial growth. Aliquots (1 ml) of a stock solution (75 mg ml^{-1}) of BP dissolved in chloroform were added to empty pre-sterilized Erlenmeyer flasks, and the solvent was allowed to evaporate. Then

1 g of sediment samples were used to inoculate 75 ml of filter-sterilized lake water (from the same lake where sediment was collected) in biphenyl-containing flasks. An additional culture flask was set up for each sample with no added carbon source to serve as a negative control. Enrichments were then incubated for 30 days at 4°C.

Bacterial isolation

Aliquots (100 µl) of the enrichments were plated on solidified Bushnell Haas (BH; Difco) (containing agar; 1.5%, wt/vol). BP was added as crystals in the Petri dish lid after inoculation. Replicate plates were incubated at 4 °C for 30 days. BH plates without BP were used as a control. Colonies were randomly selected from agar plates, picked, and subcultured three times under the same conditions for bacterial isolation.

Bacterial growth in the presence of Aroclor 1242

Bacterial growth in the presence of PCBs was tested following the protocol reported by Michaud et al. (2007). Briefly, cultures were set up in a liquid BH medium. The Aroclor 1242 mixture (Sigma-Aldrich; 100 ppm in dichloromethane) was added as the sole carbon and energy source (final concentration 0.1%, wt/vol). Cultures were incubated in duplicates at 4 °C in continuous shaking (400 rpm) for one month. The ability of the bacteria to utilize PCBs as growth substrates was assessed based on the degree of turbidity or the formation of cellular aggregates (flocs) in the test tubes. Uninoculated medium was concurrently incubated as a negative control.

16S rRNA gene amplification

188 Bacterial isolates able to grow in the presence of PCBs as the sole carbon source were identified by
189 the 16S rRNA gene sequencing, following the protocol reported by Papale et al. (2017) with some
190 modifications. Shortly, single colonies of each strain were lysed by heating at 95 °C for 10 min.
191 Amplification of 16S rRNA gene was performed with a thermocycler (Mastercycler GeneAmp PCR-
192 System 9700, Applied Biosystem, USA) using Bacteria-specific primers 27F (5′-
193 AGAGTTTGATC(AC)TGGCTCAG–3′) and 1492R (5′-TACGGYTACCTTGTTACGAC-3′). The
194 reaction mixtures assembled at 0 °C and were composed by 1 µl DNA, 0.4 µl of each of the two
195 primers (10 µM), 0.4 µl of each dNTP (10 mM), 2 µl of reaction buffer 10 ×, 0.4 µl of BSA (3 mg
196 ml− 1), 0.2 µl of *Taq* polymerase My*Taq* (5 U µl− 1), and sterile Milli-Q water to a final volume of
197 20 µl. Negative controls for DNA extraction and PCR setup (reaction mixture without a DNA
198 template) were also used in every PCR run. 1) 95 °C for 1 min; 2) 35 cycles at 95 °C for 30 s, 52 °C
199 for 30 s and 72 °C for 30 s 3) 72 °C for 10 min. The results of the amplification reactions were
200 analyzed by agarose gel electrophoresis (1%, wt/vol) in TAE buffer (0.04 M Tris-acetate, 0.02 M
201 acetic acid, 0.001 M EDTA), containing Sybr Safe (1µl/25ml final concentration) (Invitrogen).

202

203 *Sequencing and analysis of 16S rRNA genes*

204 The amplified 16S rRNA gene products were purified using the QIAquick PCR purification kit,
205 following the manufacturer's instructions. The sequencing was conducted at the Eurofins Laboratory
206 (Milano, Italy). Sequences were then red and corrected manually using FinchTV version 1.4. Next
207 relatives of isolates were determined by comparison sequences in the NCBI GenBank and the EMBL
208 databases using BLAST, and the “Seqmatch” and “Classifier” programs of the Ribosomal Database
209 Project II (http://rdp.cme.msu.edu/) (Altschul *et al.,* 1997).

210 Sequences were further aligned using the program Clustal W (Thompson et al., 1994) to the most
211 similar orthologous sequences retrieved from database. Each alignment was checked manually,
212 corrected and then analyzed using the Neighbour-Joining method (Saitou and Nei, 1987) according

213 to the model of Jukes-Cantor distances. A phylogenetic tree was constructed using the MEGA 11
214 (Molecular Evolutionary Genetics Analysis) software (Tamura *et al.,* 2021). The robustness of the
215 inferred trees was evaluated by 1000 bootstrap re-samplings.

216

217 *Screening for the presence of the bphA gene*

218 Strains showing the ability to grow in the presence of Aroclor 1242 as the sole carbon and energy
219 source were screened for the presence of the catabolic gene *bph*A involved in PCB degradation. The
220 presence of genes was highlighted by a modified protocol of Papale et al. (2017), through PCR, using
221 specific primers selected by previous analyses: 2BPHFWD1 (5' ADVCCSCGBGCCGCBTCHTCG
222 3') and 2BPHREV1 (5' ADVCCSCGBGCCGCBTCHTCG 3') (Master and Mohn, 2001). The
223 reaction mixtures were assembled at 0°C and contained 1 µl DNA, 0.5 µl of each of the two primers
224 (10µM), 2.5 µl of reaction buffer 10x, 0.025 µl of BSA (2.5%), 0.2 µl of *Taq* polymerase Bioline
225 (5U/µl), and sterile Milli-Q water to a final volume of 10 µL. Negative controls for DNA extraction
226 and PCR setup (reaction mixture without a DNA template) were also used in every PCR run. The
227 PCR program was as follows: 1) 95°C for 5'; 2) 35 cycles at 95°C for 45 '', 58°C for 1' and 72°C for
228 2'; 3) 72°C for 10'. The results of the amplification reactions were analyzed by agarose gel
229 electrophoresis (2%, w/v) in TAE buffer (0.04 M Tris-acetate, 0.02 M acetic acid, 0.001 M EDTA),
230 containing 1 µl of syber-safe solution (Fermentas) each 25 ml of gel.

231

232 *Biodegradation efficiency*

233 PCB-oxidizing isolates harboring the *bph* A gene were tested for the degradation of Aroclor 1242.
234 According to Papale et al. (2017), isolates were grown in 50 ml of Aroclor 1242-supplemented (1
235 wt/v) BH and incubated at 4 °C for 1 month. Uninoculated controls were incubated in parallel to
236 monitor the abiotic losses of the substrates. After incubation, biodegradation activity was stopped by

237 acidifying cultures with HCl 10 M to achieve pH 2. Before PCB extraction, the cultures were
238 centrifuged at 4 °C for 20 minutes at 8000 rpm to remove the excess of cellular material. After 5 µl
239 of octachloronaphthalene (OCN) solution (5 mg/ml in CH_2Cl_2) was added to each aliquot to monitor
240 substrate losses during the extraction procedure. Cultures were extracted three times with 10 ml of
241 CH_2Cl_2 in a separatory funnel to isolate the organochlorine molecules from the enrichment cultures.
242 Funnels were vigorously shaken for 15 min during each extraction. The three organic phases were
243 pooled, and the solvent evaporated to dryness.
244 The composition of PCBs and their concentration were determined by high resolution gas
245 chromatography-mass spectrometry (GC-MS), using an 7890A-5975C (Agilent Technologies) equipped
246 with a 60-m HP-5MS column (0.25 mm I.D., 0.25 µm; Agilent Technologies, Avondale, USA). Helium
247 was used as carrier gas (1.2 ml min^{-1}). Two microlitres portion of each sample were injected in
248 splitless mode. The injector was kept at 290 °C and the Transfer Line at 300 °C; the column oven was
249 initially kept at a temperature of 90 °C for 1 min, and then heated up to 150 °C at a rate of 20 °C min^{-1}
250 , and then to 300 °C at 4 °C min^{-1}, with post run at 315 °C (15 min). Analyses were performed in
251 SIM mode using octachloronaphtalene (OCN) as procedural internal standard. Crude concentrations
252 were corrected using instrumental response factors.

253

254 *Statistical analyses*

255 Principal component analysis was performed using the factoextra R package to visualize the
256 relationships between the chemical parameters measured in the natural sediment and water samples.
257 The PCA was conducted on all PCBs identified in the samples to determine the key factors underlying
258 the observed variation in the dataset. Redundancy analysis, as implemented in the vegan package of
259 R, assessed the relationship between the PCB congener profiles, sampling sites, and matrix (water
260 and sediments).

261

Results

PCB concentrations in natural samples

In the Arctic lakes, PCB concentrations in water were generally low, with limited accumulation observed for congeners such as PCB31 + 28 and PCB99, primarily detected in Solvannet (L1), while other lakes, such as Knudsenheia (L3) and Storvatnet (L4). showed negligible values. Instead, sediments showed higher levels of PCB110, PCB118, and PCB138+158, particularly prominent in Solvannet (L1). In Antarctic lakes, particularly on Livingston Island, PCB concentrations in water were slightly higher than those in the Arctic, with PCB31 + 28, PCB74, and PCB52 being the predominant congeners, evenly distributed across lakes Sofia (LS) and Argentina (LA). In sediments, significant accumulation of PCB110 and PCB74 was observed, with similar concentrations between the two lakes. At Deception Island, both water and sediments exhibited the highest PCB levels among Antarctic lakes, with peaks of PCB99, PCB151, and PCB31 + 28 in water, and notable accumulation of PCB118, PCB138+158, and PCB110 in sediments. In water, Extremadura (LE) Lake showed the highest concentrations of PCB99 and PCB151. Crater (LC) Lake also displayed relatively high concentrations, particularly for PCB31 + 28 and PCB81. In sediments, Zapatilla (LZ) Lake and Telefon (LT) Lake demonstrated elevated levels of PCB110 and PCB138+158, while Ballaneros (LB) Lake showed consistent but lower concentrations across most congeners. Comparing the Arctic and Antarctic regions, sediments, in both areas, acted as key sinks for PCBs, but Antarctic lakes evidenced greater diversity and higher concentrations in both water and sediments, particularly at Deception Island.

The PCA of PCBs retrieved in the Arctic showed a sharp separation between water and sediments (fig. 3a). Specifically, water samples from Solvannet (L1), Storvatnet (L4), and Tvillingvatnet (L5) lakes correlated with PCB 81, whereas sediment samples from Solvannet (L1) Lake correlated with PCB 99, PCB 151, PCB 77, PCB 123, PCB 74, PCB 149, and ∑PCB 84 + PCB 90 + PCB 101. Sediment samples from Knudsenheia (L3) and Tvillingvatnet (L5) lakes correlated with PCB 52,

287 ∑PCB 31+28, PCB 110, ∑PCB 84 + PCB 90 + PCB 101, ∑PCB 153 + PCB 168, and PCB 118. With
288 respect to Antarctic samples, water from Extremadura (LE), Ballaneros (LB), and Telefon (LT) lakes
289 correlated with PCB 123, PCB 151, PCB 99, PCB 77, ∑PCB 31 + PCB 28, PCB 105, and PCB 81,
290 while no correlations were found for the water from Crater (LC), Zapatilla (LZ), Argentina, and Sofia
291 (LS) lakes (fig. 3b). The sediments from Sofia (LS), Argentina (LA), Telefon (LT), and Ballaneros
292 (LB) lakes correlated with ∑PCB 138 + PCB 158, PCB 149, PCB 74, PCB 52, PCB 110, PCB 118,
293 ∑PCB 155 + PCB 168, and ∑PCB 84 + PCB 90 + PCB 101.

294 Comparing the results of both the Arctic and Antarctica (fig. 3c), the PCA highlighted that the
295 sediment of Solvannet (L1) Lake was completely separated from the others and that there was no
296 clear separation between the two poles or between the matrices. In the first quadrant, we find water
297 samples from Arctic lakes Knudsenheia (L3), Storvatnet (L4), and Tvillingvatnet (L5), as well as
298 Glacier (L2) Lake water and sediment. In the fourth quadrant, we find water from Sofia (LS) and
299 Solvannet (L1) Lakes. Additionally, Arctic sediments from Storvatnet (L4) and Knudsenheia (L3),
300 plus Crater (LC) Lake sediment, are in the first quadrant. Nearby, the second quadrant contains
301 sediment from Tvillingvatnet (L5) and other Antarctic lakes. The third quadrant includes water from
302 Antarctic lakes Ballaneros (LB), Extremadura (LE), Telefon (LT), and Crater (LC). Lastly, the fourth
303 quadrant also has water from Antarctic lakes Zapatilla (LZ) and Argentina (LA) together.

304

305 *Isolation, screening of bphA gene, and phylogenetic identification of PCB-oxidizing bacterial*
306 *strains*

307 A total of 46 Arctic strains were isolated from the biphenyl enrichments, i.e., 16 strains from
308 Solvannet Lake (L1), 14 from Glacier Lake (L2), 7 from Knudsenheia Lake (L3), and finally 9 from
309 Storvatnet Lake (L4). Among these, 24 strains grew in the presence of Aroclor 1242, with 18
310 harbouring the *bph*A gene. These 18 strains were identified by the 16S rRNA gene sequencing (table
311 2). The majority of the isolates (15) belonged to the Gammaproteobacteria within the genus

312 *Pseudomonas*. A unique isolate belonged to the Betaproteobacteria, within the genus
313 *Janthinobacterium*. The affiliation could not be determined for two strains (S3D-1 and S3D-2).

314 A total of 66 Antarctic strains were isolated from the enrichments, distributed as follows: 14 from
315 Sofia Lake (LS), 16 from Argentina Lake (LA), 8 from Crater Lake (LC), 8 from Telefon Lake (LT),
316 and 12 from Ballaneros Lake (LB). No strains were isolated from Extremadura Lake. Among these,
317 22 bacterial isolates were able to grow in the presence of Aroclor 1242. The *bph*A gene was detected
318 in seven of them, which were all identified as affiliates to the genus *Pseudomonas* (phylum
319 Gammaproteobacteria) (table 2). The affiliation could not be determined for four strains (ATD-2,
320 ATD-3, ATD-5 and ATD-9)

321 Antarctic isolates are part of the Italian Collection of Antarctic Bacteria of the National Antarctic
322 Museum (CIBAN-MNA), kept at the University of Messina (voucher codes MNA-CIBAN-2276 to
323 MNA-CIBAN-2282; Supplementary Table S1).

324

325 *Biodegradation efficiency*

326 Based on the tests described above, seven *Pseudomonas* isolates from Arctic lakes were selected to
327 assess their PCB degradation capability on Aroclor 1242. Two strains, namely S3D-2 (not identified)
328 and *Pseudomonas* sp. S3D-5, both from Lake Knudsenheia, were particularly efficient (fig. 4a).

329 When examining specific PCB groups, a slight increase in monochlorinated PCBs, particularly PCB-
330 1, was observed in comparison to the control (fig. 5 a). PCB-11 levels increased in all cultures except
331 for *Pseudomonas* sp. 3D-5, in which its amount was as in the control (fig. 5 b). For trichlorinated
332 PCBs, the formation of PCB-16, absent in the control, was detected in cultures of *Pseudomonas* spp.
333 S1D-8 and S2D-11, and S3D-1 (not identified) (fig. 5 c). However, *Pseudomonas* sp. S2D-14 culture
334 showed no traces of PCB-16 but was unique in forming PCB-18. Tetrachlorinated PCBs was the most
335 degraded, with complete removal by *Pseudomonas* spp. (strains S1D-9, S2D-14), and S3D-2 (not

identified) (fig. 5 d). The other bacterial cultures evidenced only small residual amounts compared to the control. In contrast, pentachlorinated PCBs increased in all cultures except for that of *Pseudomonas* sp. S3D-5, where levels remained unchanged from the control, despite partial transformation (fig. 5 e). However, the overall levels of pentachlorinated PCBs across all bacterial cultures were minimal with respect to the total. Finally, small amounts of hexachlorinated PCBs were detected in cultures of *Pseudomonas* spp. S1D-8 and S1D-9 (fig. 5 f), while nonachlorinated PCBs were detected in *Pseudomonas* sp. S3D-5 culture (fig. 5 g). Strain S3D-2 (not identified) emerged as the most efficient when considering degradation across all PCB categories.

All *bph*A-harboring Antarctic isolates (7) were tested for PCB degradation tests on Aroclor 1242. In general, many strains showed high degradation efficiency, with *Pseudomonas* YAAD-5 affiliated to and ATD-2 (not identified) were the most efficient (fig. 4b). Specifically, when analysing by category, a slight increase in monochlorinated compounds compared to the control was observed in all bacterial cultures except for ATD-2, which showed values like the control; PCB-1 was the most prevalent compound in this category, present in all bacterial cultures (fig. 6a). Among dichlorinated compounds, some cultures, such as those of *Pseudomonas* spp. ATD-5 and ASD-5, exhibited higher quantities than the control, while others showed values comparable to it, with PCB-11 being the most abundant molecule (fig. 6b). For trichlorinated compounds, *Pseudomonas* spp. ATD-9 and ASD-5 cultures displayed significantly higher values than the control, while other cultures, including those of *Pseudomonas* sp. YAAD-5 and ATD-2 (not identified), had values only slightly higher or comparable (fig. 6c). In contrast, tetrachlorinated PCB were found below control levels in all bacterial cultures, and in two cases (i.e. ATD-2 and YAAD-6) they were not detected at all (fig. 6d). For pentachlorinated PCBs, the strains ATD-2 and *Pseudomonas* sp. YAAD-5 showed values like the control, while the other cultures had higher levels (fig. 6e). Finaly, small amounts of hexachlorinated compounds were detected in *Pseudomonas* sp. ASD-5, and heptachlorinated compounds were identified in *Pseudomonas* sp. YAAD-6 cultures. ATD-2 and *Pseudomonas* sp. YAAD-5 emerged as the most efficient strains across all PCB categories.

362

363 **Discussions**

364 The results of PCB concentrations retrieved across all analyzed samples, both in Arctic and Antarctic
365 regions, showed higher concentrations in Antarctic lakes compared to Arctic lakes and in sediments
366 compared to water. Previous studies have reported that PCBs tend to accumulate in sediments due to
367 their low solubility in water and high lipophilicity, making sediments long-term reservoirs for these
368 contaminants (Cabrerizo et al., 2018; Blais et al., 2001; Malmquist et al., 2003). The predominance
369 of heavier chlorinated congeners, such as tetrachlorobenzene and hexachlorobenzene, in sediments
370 reflects their lower volatility and more remarkable environmental persistence (Jönsson et al., 2002).
371 Conversely, lighter congeners like monochlorobenzene and dichlorobenzene were more prevalent in
372 water samples (Dewulf & Langenhove, 1997). These findings align with the global fractionation
373 model, which predicts preferential atmospheric transport and deposition of less-chlorinated PCB
374 congeners at higher latitudes (Muir et al., 1996; Malmquist et al., 2003). Interestingly, water samples
375 collected at Deception Island, especially for congeners like PCB99 and PCB151, presented slightly
376 elevated concentrations compared to those reported by Vecchiato et al. (2015) in other Antarctic
377 regions, suggesting possible localized inputs or increased deposition in volcanic environments. The
378 variability observed among the lakes at Deception Island highlights the significant role of local
379 factors, such as sediment composition and hydrology, in influencing PCB distribution. This is
380 consistent with Borghini et al. (2005), who emphasized the importance of site-specific dynamics in
381 contaminant behavior. In contrast, Arctic lakes displayed lower PCB concentrations in water, while
382 sediment levels were comparable to those recorded in Arctic glacial marine and freshwater settings.
383 (Pavlova et al. 2023, Pizzini et al., 2023, Bizzotto et al., 2008).

384 Evaluation of polychlorinated biphenyl degradation capabilities in bacterial isolates from Arctic and
385 Antarctic lakes revealed notable outcomes. Of the 46 strains tested from Arctic lakes, 24 exhibited
386 positive PCB degradation results, with 18 of them harboring the *bph*A gene. Similarly, 22 out of 66

Antarctic strains tested positive, with the *bph*A gene detected in 15 isolates. This suggests a higher proportion of PCB-degrading bacteria in Arctic environments compared to Antarctic samples, potentially reflecting regional differences in environmental conditions and selective pressures. The observed results exceeded the proportions (approximately 10% higher) previously reported in the same environments, though not specifically for lake samples (Master & Mohn, 1998; Michaud et al., 2007). These findings align with the work of Papale et al. (2022) on Antarctic lakes, indicating that these aquatic habitats serve as ideal environments for the proliferation of PCB-degrading bacteria due to the accumulation of these persistent organic pollutants from both marine and terrestrial sources in the surrounding regions.

The analysis of bacterial strains possessing the *bphA* gene revealed a predominance of *Pseudomonas* members among Gammaproteobacteria. Among the Arctic isolates, common species included *P. fluorescens*, *P. gessardii*, and *P. jessenii.* Similarly, Antarctic strains comprised *P. antarctica*, *P. fragi*, and *P. migulae*. The prevalence of these cold-adapted taxa has been previously documented (Master & Mohn, 1998; Bopp, 1986; Shuai et al., 2016) and underscores their potential for PCB biodegradation applications. PCB degradation assays revealed that Arctic strains generally demonstrated higher degradation efficiency across various PCB categories compared to Antarctic strains. Notably, Arctic strain S3D-2 (filiated with the genus *Pseudomonas*) and Antarctic strain ATD-2 (not identified) emerged as the most effective across all PCB congeners. Analyzing specific PCB categories, dichlorinated and trichlorinated compounds exhibited varying degradation patterns. For instance, Arctic strains like S3D-2 showed efficient degradation of PCB-16, while Antarctic strain ATD-9 demonstrated partial degradation with notable byproduct formation. Tetrachlorinated compounds were universally well degraded, with Arctic strains such as S1D-9 and Antarctic strains like YAAD-5 showing near-complete removal. These findings align with the known capabilities of *Pseudomonas* to utilize biphenyl as a carbon source through the biphenyl dioxygenase pathway, encoded by the *bphA* gene, which initiates PCB breakdown (Bopp, 1986; Furukawa and Fujihara, 2008; Michaud et al., 2007). Obtained results demonstrate the significant potential of cold-adapted

bacteria, particularly those from the genus *Pseudomonas*, in degrading PCBs in polar environments. The prevalence of the *bphA* gene and the associated metabolic pathways suggest that these strains are well-adapted to PCB degradation even under extreme conditions. Future studies should focus on characterizing the enzymatic pathways involved in PCB degradation, with particular attention to the *bphA* gene cluster and its role in biphenyl oxidation. Additionally, bioaugmentation strategies using these strains in PCB-contaminated polar environments could mitigate the environmental impact of PCB pollution. Given the "global distillation" phenomenon, which concentrates PCBs in polar regions due to their volatility and persistence (Furukawa & Fujihara, 2008), applying cold-adapted bacteria like *Pseudomonas* offer a promising avenue for bioremediation. Moreover, studies such as those by Papale et al. (2017), underline the importance of understanding biodiversity patterns and ecological interactions to inform effective PCB remediation approaches in extreme environments.

Conclusions

This study underscores the critical significance of Arctic and Antarctic lakes in the global dynamics of polychlorinated biphenyls, as they serve as both reservoirs and potential sites for bioremediation. The distinct chemical profiles observed between the two regions, shaped by environmental and geographical factors, highlight the influence of global distillation and selective deposition of PCB congeners. Notably, Arctic lakes exhibited a higher proportion of PCB-degrading bacterial strains, with a notable prevalence of species from the genus *Pseudomonas*, which harbored the *bph*A gene and demonstrated substantial degradation capabilities across various PCB categories. Conversely, Antarctic lakes, despite their lower overall PCB concentrations, also hosted efficient PCB-degrading strains, such as ATD-2, showcasing the resilience and adaptability of cold-adapted microbial communities. The findings underline the synergy between chemical persistence and biological degradation pathways in polar environments. The predominance of heavier chlorinated congeners in sediments and the efficiency of microbial degradation, particularly for tetrachlorinated PCBs, underscore the potential of leveraging these cold-adapted bacterial strains for bioremediation. Strains

such as S3D-2 and ATD-2 represent promising candidates for future applications to mitigate PCB pollution in these fragile ecosystems. Ultimately, this study contributes to a deeper understanding of the complex interactions between PCB dispersal, environmental accumulation, and the adaptive potential of indigenous microbial communities in the Arctic and Antarctic regions.

CRediT authorship contribution statement

RAC: Investigation, Formal analysis, Writing-Reviewing and Editing, Data curation, Conceptualization; RC: Investigation, Reviewing and Editing; GS: Formal analysis, Reviewing and Editing; VM: Formal analysis, Reviewing and Editing; LA: Supervision, Methodology, Writing-Reviewing and Editing; MA: Formal analysis; MP: Supervision, Methodology, Writing-Reviewing and Editing, Funding Acquisition, Data Curation, Conceptualization.

Data availability

Nucleotide sequences have been deposited in the NCBI GenBank database under the accession numbers PQ686975-PQ686991 and PQ686993- PQ686998.

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Declarations of competing interests

Angelina Lo Giudice declares her involvement as an Editorial Board Member of Polar Biology. The other authors declare no competing interests.

464

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Estratto per riassunto della tesi di dottorato

L'estratto (max. 1000 battute) deve essere redatto sia in lingua italiana che in lingua inglese e nella lingua straniera eventualmente indicata dal Collegio dei docenti.

L'estratto va firmato e rilegato come ultimo foglio della tesi.

Studente: Alessandro Ciro Rappazzo matricola: 956564

Dottorato: Polar Science

Ciclo: 36

Titolo della tesi[1] : Microbial response to human pollutants in polar lakes

Abstract:

Lakes are sensitive ecosystems in the Arctic and Antarctica. They host diverse microbial communities. Minor changes in snow and ice cover or the introduction of pollutants can drastically impact lake ecology. The present study evaluated bacterial communities in polar lakes, which showed little variation between polar regions, and the ability of microbial communities to tolerate and degrade pollutants. Arctic metal-tolerant strains were mainly Gammaproteobacteria, whereas Antarctic strains were predominantly Actinobacteriota. Heavy metal tolerance tests showed moderate abilities, with greater tolerance to iron followed by mercury and copper. PCB degradation tests revealed nearly equal oxidation abilities, with the *bphA* gene found in most identified strains, primarily from *Pseudomonas*. Degradation efficacy was high across both poles. Overall, these findings enhance our understanding of polar bacteria's biotechnological potential for environmental remediation.

I laghi sono ecosistemi sensibili, specialmente nelle regioni polari, che ospitano comunità microbiche diversificate. Anche piccoli cambiamenti possono influenzare drasticamente l'ecologia dei laghi. Questo studio ha valutato le comunità batteriche nei laghi polari e la capacità delle comunità microbiche di tollerare e degradare gli inquinanti. Le comunità hanno mostrato una modesta variabilità. I ceppi artici che tolleravano i metalli erano principalmente Gammaproteobacteria, mentre quelli antartici erano prevalentemente Actinobacteriota. È stata evidenziata una maggiore tolleranza al ferro, seguita da mercurio e rame. Per i PCB era evidente la capacità di ossidazione per entrambi i siti, ed i ceppi erano principalmente appartenenti al genere *Pseudomonas.* L'efficacia della degradazione è risultata elevata in entrambe le regioni polari. Nel complesso, questi risultati migliorano la comprensione del potenziale biotecnologico dei batteri polari per la bonifica ambientale.

Firma dello studente

Alessandro Ciro Rappazzo

[1] Il titolo deve essere quello definitivo, uguale a quello che risulta stampato sulla copertina dell'elaborato consegnato.