UNIVERSITÀ DEGLI STUDI DI MESSINA

DIPARTIMENTO DI SCIENZE CHIMICHE, BIOLOGICHE, FARMACEUTICHE ED AMBIENTALI

DOTTORATO DI RICERCA

IN BIOLOGIA APPLICATA E MEDICINA SPERIMENTALE

XXXV CICLO

SSD BIO/19

Assessment of fungal diversity present in Arctic and Antarctic lakes and selection of Heavy Metal tolerant fungal isolates

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Anno Accademico 2021/2022

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1 ABSTRACT

Lakes are prominent features of the Arctic landscape and are also common in many parts of Antarctica. Various microbial communities (especially bacteria) have been characterized, including those in Antarctic and Arctic lakes, which face different extreme conditions such as low temperatures, high salinity, pH variation, seasonally high UV radiation, and low nutrient availability. However, the fungal component is often overlooked. Compared to other environments, studies on fungal communities in polar lakes are scarce, and most of them applied traditional culturing methods, which do not reveal the full diversity of the resident mycobiota. Furthermore, Arctic and Antarctic areas are generally considered pristine and unexplored environments, even if the presence of persistent pollutants, such as heavy metals, has been recorded many times. Lake ecosystems, especially small lakes and ponds are very sensitive to environmental perturbations. Although, microorganisms living in cold environments, including fungi, have been shown to cope with such pollutants.

In this context we decided to assess the diversity of fungal communities present in water and sediments collected from Arctic lakes in Ny-Ålesund (Svalbard Island, High Arctic) and from Antarctic lakes in Deception and Livingston Island, using cultural methods and DNA metabarcoding. Furthermore, the study focused on the analysis of fungal cultivable fraction able to tolerate heavy metals (HMs) (iron, copper and mercury). Our results showed a total of 5980 amplicon sequence variants (ASVs), and only 102 (1.7 %) were shared between the two Polar regions. For Arctic lakes, unknown fungi dominated the sequence assemblages, suggesting the dominance of possibly undescribed fungi. The phylum Chytridiomycota was the most represented in all Arctic and Antarctic lakes, followed by Rozellomycota, Ascomycota, Basidiomycota, and the less frequent Monoblepharomycota, Aphelidiomycota, Mortierellomycota, Mucoromycota and, Neocallimastigomycota. At the genus level, the most abundant genera were psychrotolerant and cosmopolitan cold-adapted fungi including

Alternaria, *Cladosporium*, *Cadophora*, *Ulvella* (Ascomycota), *Leucosporidium* and *Vishniacozyma* (Basidiomycota), and *Betamyces* (Chytridiomycota). The assemblages displayed high diversity and richness. The assigned diversity was composed mainly of taxa recognized as saprophytic fungi, followed by pathogenic and symbiotic fungi. A total of 51 fungal strains (31 filamentous fungi and 20 yeasts) were isolated from natural culture enrichments of sediment samples with 1000 ppm iron (Fe) and copper (Cu), and 100 ppm of mercury (Hg). Among them, 12 and 1 strains were able to grow up to 5000 ppm of Fe and Cu, respectively and 5 strains grew up to 500 ppm of Hg. A total of 7 strains were able to tolerate two different metals. Heavy metal tolerant isolates were assigned to the genera *Pseuderotium* sp., *Rhodotorula* sp., *Cryptococcus* sp. and to the species *Glaciozyma antarctica*, *G. watsonii*, *G. martini*, *Cadophora fastigiata*, *Holtermanniella wattica* and *Mrakia robertii*, already widely recognized as HMs tolerant, thus showing that fungi in these areas also have a significant role in environmental decontamination.

2 INTRODUCTION

The Polar regions (Arctic and Antarctic) occupy about 14% of the landmass, and constitute the largest portion of the cryosphere, the part of Earth's surface where water exists in a solid state. The Arctic has an area of about 7.1 million km^2 (4.8% of the Earth's surface), including the Arctic Ocean and adjacent seas (Nordic Sea and Bering Sea) as a major component (2/3 of the region), continental lands, consisting in the northern fringes of the Northern Hemisphere continents (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 Southern Greenland (Denmark) that is the world's largest island. The mean annual temperature is around −18 °C (Barry et al., 2013; Tsuji & Hoshino, 2019). Antarctica is a large continental landmass overlying the geographic South Pole and has an area of approximately 14 million km^2, around 10% of the Earth's surface. From a biogeographic point of view, the most accepted classification scheme divides Antarctica into two regions: continental Antarctica and maritime Antarctica (Convey et al., 2014; Singh et al., 2015). The former region is located in the innermost part of the continent, characterized by high latitude and a layer covered with ice reaching a height of more than 4250 m, which results in the most rigorous living conditions on the continent. The latter region includes a clearly defined maritime region that encompasses the Antarctic Peninsula and the associated islands of the Scotia Arc and extends from the South Sandwich Islands through South Orkney and South Shetland Islands and down the western side of the Antarctic Peninsula to approximately 72°S (Camacho, 2006). It has longer and warmer summers than the mainland, as well as marine influences (Bolter et al., 2002). Antarctica is the fifth largest continent, with about 99.6–99.8% of its area permanently covered by ice and snow. It holds ~90% of the ice on Earth, which is equivalent to ~60–70% of the available freshwater on the planet. Only 1–3% of the Antarctic surface is free of ice in summer months, and most of these areas are rocky deserts and rocky ice-covered mountains (Yergeau et al., 2007; Singh et

al., 2018). Mean monthly air temperatures in coastal areas range from 2–3°C to −35 °C, over the year, with those in inland continental regions ranging between −25°C in summer and −70°C in winter (Ravindra & Chaturvedi, 2011; Convey, 2017; Tsuji & Hoshino, 2019). In winter the surface of the Southern Ocean surrounding Antarctica freezes, doubling the surface area of the continent, and further increasing its isolation. Such isolation has important implications for climate, colonization and biodiversity.

2.1 ARCTIC LAKES

A major feature of Arctic landscapes is the large number of lakes and ponds, which in some regions can cover up to 90% of the total surface area (Raatikainen & Kuusisto, 1990; 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" (Kling, 2009). 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 fresh water to migratory nesting birds, resident animals, and humans (Rautio et al., 2011). In general, the distribution of lakes and ponds in the Arctic is controlled by the climate, geomorphology, substrate permeability, glacial history, and the presence of permafrost. The lakes can be located 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) (Jeppessen et al., 2021). Thermokarst lakes and ponds (usually shallow, formed by thawing ice-rich permafrost) are the most frequent aquatic ecosystem type in the Arctic, which often generate a complex of waterbodies that are hot spots of biological activity, with abundant 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. However, climate change has had a significant impact on Arctic lakes, including the duration of ice cover, the temperature, and the amount, type, and timing of precipitation. As a result of the rising temperature, new lakes will appear due to glacier retreat and the thawing of permafrost. Some lakes may also change from glacier affected turbid lakes to isolated clear-water lakes or disappear when they are no longer dammed by ice or cut off from glacier runoff water. Arctic lakes are typically oligotrophic, or even ultraoligotrophic (Hamilton et al., 2001; Michelutti et al., 2002) and with the exception of eutrophication caused by ancient whaling residues (Douglas et al., 2004), sewage and wastewater (Kashulin et al., 2021), and more recently by increased geese and seabird populations (Michelutti et al., 2009; Hessen et al., 2017; Jensen et al., 2019), eutrophic lakes are rarely mentioned in the Arctic scientific literature.

2.2 ANTARCTIC LAKES

The Antarctic continent and sub-Antarctic islands represent some of the most diverse and interesting lake districts on the planet (Hodgson, 2012). The majority of lakes are found in the 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 (Vicent et al., 2008; Sokratova et al., 2011; Phartiyal et al. 2011; de Souza et al., 2021; Gonçalves et al., 2012; Ogaki et al., 2019). Most Antarctic lakes are formed when the ice recedes and because of exposed depressions in the terrain, formed either by glacial erosion or by the deposition of terminal moraines, or because of folds and depressions in the underlying geological topography (Hodgson, 2012). Water temperature generally has values < 10°C and in winter ice cover

between 1.5 and 6 m thick acts as a thermal barrier that prevents the freezing of the lake bottom (Hodgson, 2012). The thick perennial ice cover can minimize wind-generated currents, limit lake circulation, restrict light penetration, increase dissolved gas concentrations, and cause heterogeneous sedimentation on the lake bottom (Doran et al., 2000; Phartiyal et al., 2011). Regarding physicochemical features, Antarctic 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, 2012). 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 (Butler, 1999; Quayle & Convey, 2006; Gonçalves et al., 2012; Hodgson, 2012; de Souza et al., 2021; Ogaki et al., 2019). Typically, the freshwater bodies of Antarctica are in direct contact with rocks, soil and mud, and their catchments include vegetation such as *Deschampsia antarctica*, *Colobanthus quitensis* (these flowering plants in the maritime Antarctic only), mosses, macroalgae and lichens (Ogaki et al., 2019). However, despite their biota being primarily microbial, what these Antarctic lake biota lacks in size is more than compensated for in biomass and species diversity (Vincent, 2000; Hodgson, 2012). 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 et al., 2012). They are typically shallow (< 10 m deep), transparent, receive high light and ultraviolet radiations, 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. in Fildes Peninsula in King George Island. In these areas the climate is less extreme than the interior of the continent, so that in summer, although temperatures below 0 C predominate, ice melting and liquid precipitation are common, which led to a more active hydrological cycle with sediment and nutrient circulation throughout the lacustrine basin (Quayle et al., 2002; Mckay et al., 2009; Camacho et al., 2012). In maritime Antarctica most lakes originated from glacial

retreat, and a few have been formed by tectonic activity and by volcanic activity (Priddle & Heywood, 1980). In fact, some lakes, such as the Lake Crater 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 South Shetland Islands and in Lake Kroner on Deception Island, fumarole activity supplies heat to the lake, which makes it a unique geothermally heated lake in Antarctica (Priddle & Heywood, 1980). Most of the lakes in continental Antarctica show a typical concentration of ions and solutes, that is acquired when glacial meltwaters come in contact with soils and sources that surround them, making them saline or hypersaline (Green & Canfiel, 1984; Priscu & Foreman, 2009). 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 (Laybourn-Parry & Wadham 2014; Shevnina & Kourzeneva, 2017). The lakes and ponds found in these areas are shallow and relatively warm, but there are also some large, deep, and cold-water lakes, that are generally covered with ice, 3–5 m thick, throughout the year (Matsumoto 1993; Doran et al. 1996). They occur in tectonic faults, depressions of valleys or in proximity to glaciers (Sokratova et al., 2011; Shevnina & Kourzeneva, 2017). High-density stratification and hypersaline bottom water, probably formed during glacial lowstands, are typical of most of these lakes. 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 et al., 2011). Nutrients are concentrated in the anoxic bottom waters because of the lack of circulation, producing an oligotrophic status in the lakes and ponds (Bishop et al., 2014). 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., 2014). 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 (Shevnina & Kourzeneva, 2017) are located in Larsemann Hills, that consists of several ice-free peninsulas and islands along the coast; few perennially ice-covered lakes at Bunger Hills (Doran et al., 2000), 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 around the whole 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, active and partially interconnected subglacial hydrological systems connecting more than 379 lakes exist beneath the Antarctic ice sheet (Wright & Siegert, 2012). These lakes lie up to 4200 m under the Antarctic ice sheet, range in size from 1 to 241 km long (Hodgson et al., 2004) and are subject to high pressure (approximately 350 atmospheres), low temperatures (about −3 °C) and permanent darkness, characteristics that makes 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).

2.3 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. Four characteristics of the physical environment distinguish polar aquatic ecosystems from those at lower latitudes: persistent cold-water temperatures; freeze-thaw cycles; prolonged ice-cover; and the extreme seasonality of solar radiation and temperature regimes. Such extreme conditions limit the distribution and the abundance of organisms to produce aquatic ecosystems that are dominated by microscopic species. However, given the proximity to the north-temperate zone, Artic waters are characterized by much more developed food web, with more diverse animal, plant, and microbial composition than Antarctica (Vincent et al, 2008). In Antarctica, the most well-known organisms include only a few species of animals, several mosses, lichens, and two endemic angiosperms. In this context, microbial communities of viruses, archaea, bacteria, microalgae, and fungi represent the largest reservoir of biodiversity (Ogaki et al., 2019). Severe polar conditions, including low temperature, low humidity, and freezing limit together the activity of microorganisms and slow all the biogeochemical processes and also the development of vegetation, which in turn reduces the root-associated microorganisms (rhizosphere community) (Vincent et al., 2008). Polar regions host an extraordinary diversity of lake types, ranging from freshwater to hypersaline, from highly acidic to alkaline and from perennially ice-covered waters to concentrated brines that never freeze. They show different thermal regimes in summer, from fully mixed to thermally stratified and different 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). Particular lake types are exclusive from Polar regions, i.e. 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 melt water 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 microbial community composition 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 (Schmidt et al., 1991). In some 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 and 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). Lakes 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 the glacial recession. High latitude lakes have broad global significance, acting as residences of unique species and communities and as early detectors of environmental change because snow and ice cover variation markedly affect all ecological variables (Quayle et al., 2002; Margesin & Collins, 2019). In fact, multiple stressors associated with local and global human impact such as contaminant influxes, increased exposures to ultraviolet radiation and climate change have a striking impact on these aquatic ecosystems, and even small changes in their physical, chemical or biological characteristics can be amplified into major shifts in their

limnological properties and in ecosystems structure and functioning (Quayle et al., 2002, 2003; Flocco et al., 2019; Rosa et al., 2019; Ogaki et al., 2020a).

High latitude lakes are already suffering marked impacts due to climate change, including the loss of perennial ice cover leading to increases in the duration of open water conditions and water temperatures, stronger water column stratification, and changes in the balance of inflow and outflow, in some cases leading to complete drainage or drying of lakes and swamps (Quayle et al., 2002, 2003; Vincent et al., 2008). The combination of the above-mentioned features makes polar lakes interesting and unique environments to study the taxonomy and ecology of microbial communities living under extreme conditions.

2.4 MICROFUNGI INHABITING POLAR LAKES

Important among the different microbial groups present in polar lakes are the fungi. The kingdom of Fungi represents one of the most diverse groups of organisms on the planet, with an essential role in ecosystem processes and functioning. Traditionally, fungi are defined as heterotrophic, spore-bearing eukaryotes, capable of producing tubular networks made of chitin or cellulose and do not contain chlorophyll. Fungi are the biggest osmotrophic specialists, producing a plethora of secretory enzymes and obtaining nutrients through extracellular digestion and endocytosis. They can reproduce both sexually and asexually, they come in many forms from unicellular, such as yeasts and zoosporic parasites, to multicellular filamentous organisms with diverse morphologies (Alexopoulos and Mims, 1979). Thanks to extremely various metabolic strategies and high morphological diversity, fungi have conquered numerous ecological niches and have shared various interactions with other living organisms. In fact, fungi are found virtually in all environments throughout the globe (Stajich, 2017), including extreme environments, such as torrid and polar deserts (Selbmann et al., 2005; Powell et al. 2012; Gonçalves et al. 2016), hypersaline salterns (Gunde-Cimerman et al., 2000) and deep-sea

(De Leo et al., 2019; Marchetta et al., 2018). Members of the kingdom Fungi can be found living in symbiotic associations with plants, algae, insects, and animals and they can act as predators, pathogens and parasites of myriad other organisms (Naranjo-Ortiz & Gabaldón, 2019a). The global diversity of fungi has been first estimated by Hawksworth (1991) to be 1.5 million species, however, the increasing development of DNA sequencing technologies which occurred in the last ten years led to expanding the estimates of fungal species numbers to 2.2-13.2 million. Despite this high estimate diversity, to date, only around 150.000 fungal species have been described (Hyde, 2022) and are included in nine phylum clades: Opisthosporidia, Chytridiomycota, Neocallimastigomycota, Blastocladiomycota, Zoopagomycota, Mucoromycota, Glomeromycota, Basidiomycota and Ascomycota (Naranjo-Ortiz and Gabaldón, 2019b). Fungi constitute a well-founded component of terrestrial ecology due to more than 100 years of research that has highlighted their role in biogeochemical cycling and promoting biodiversity (Peay et al., 2016), while aquatic ecosystems, in contrast, were long overlooked as fungal habitats. Thanks to recent advances in DNA-sequencing technology, increasing evidence that fungi are abundant in many, if not all, aquatic ecosystems was revealed (Shearer et al., 2007; Grossart et al., 2019). However, fungal diversity, quantitative abundance, ecological function and, in particular, their interactions with other microorganisms remain mainly speculative, unexplored and missing from current general concepts in aquatic ecology and biogeochemistry (Grossart et al., 2019). To date, only a few studies have been conducted to assess fungal community diversity in Arctic lakes. The study of Zhang et al. (2016) was the first to use high-throughput sequencing technologies to comprehensively analyze fungal communities that inhabit fresh waters (streams, ponds, melting ice water, and estuaries) in the Ny-Ålesund Region (Svalbard, High Arctic), using 454 pyrosequencing with fungi-specific primers targeting the internal transcribed spacer (ITS) region of the ribosomal rRNA gene. Aquatic fungal communities in this region showed high diversity, with a total of 43.061 reads

belonging to 641 operational taxonomic units (OTUs) being found. In particular, in pond water samples, from 106 to 160 OTUs were obtained, with different dominant fungal phyla in three different lake water samples (Ascomycota, Basidiomycota and Chytridiomycota, respectively). The major orders were Helotiales, Eurotiales, and Pleosporales in Ascomycota, Holtermanniales in Basidiomycota, and Chytridiales and Rhizophydiales in Chytridiomycota. The major genera included *Penicillium*, *Rhodotorula*, *Epicoccum*, *Glaciozyma*, and *Holtermanniella* and the most commonly identified species were *Mrakia blollopis*, *Rhodotorula glacialis*, *Penicillium paneum* and *Penicillium purpurogenum*. Comeau et al. (2016) assessed the fungal diversity of Arctic aquatic environment, examining pyrosequencing libraries of the V4 region of 18S rRNA (DNA and RNA) originating from marine and freshwater samples (including three lakes in the Canadian High Arctic). Chytridiomycota represented the most common phylum in lake samples, followed by Ascomycota, Basidiomycota and Cryptomycota, with *Leucosporidium*, *Aureobasidium*, *Leptosphaeria*, *Eurotium and Leotiomycetes* as the most common genera. Perini et al. (2019) studied the composition of fungal communities in three polythermal glaciers and associated aquatic environments such as clear ice floating in seawater, glacial meltwater, moraine lake water, sea water and tap water in Kongsforden, Svalbard. A number of 174.770 reads were obtained by NGS analysis of lake water samples which mainly belonged to Ascomycota, Basidiomycota, Chytridiomycota and Rozellomycota, with Chytridiomycota predominating, followed by Basidiomycota most represented by the class Agaricomycetes. The major orders identified were Dothideales, Pleosporales, Verrucariales and Hypocreales in Ascomycota; Hymenochaetales, Polyporales, Malasseziales, Kriegeriales and Leucosporidiales in Basidiomycota; Rhizophydiomycetes in Chytridiomycota. At genus and species level, sequences were assigned to *Aureobasidium* sp., *Comoclathris sedi*, *Atla oulankaensis*, *Verrucaria alpicola*, *Lecanicillium* sp., *Meripilus giganteus*, *Libkindia masarykiana*, and *Betamyces americae-meridionalis*. Grum-Grzhimaylo et al. (2018),

investigated the diversity of flamentous culturable fungi in different parts of the brackish Lake Kislo-Sladkoe (White Sea, Russia). A total of 313 fungal isolates were obtained from the 42 samples of different types of soils around the lake and its bottom sediments. These isolates belonged to 127 taxa, of which 111 (71.2%) were Ascomycota, 8 (5.1%) Basidiomycota, and 8 (5.1%) Zygomycota. In total, 33 fungal taxa were identified in the samples collected from the lake's bottom sediment. The number of fungi from the bottom sediment in various samples varied from 4 to 7. The lake sediments harbored *Cladosporium* spp., *Talaromyces* spp., *Penicillium* spp., *Tolypocladium* spp., and *Sistotrema* sp. In particular, in lake bottom the identified species were *Acremonium* sp., *Aspergillus tubigensis*, *Cladosporium cladosporioides*, *Penicillium thomii*, *P. verruculosum*, *Sistotrema brinkmannii*, *Talaromyces funiculosum*, *T. variabilis*, *Talypocladium cylindrosporum*, *T. inflatum* and *Xylobolus* sp. In Antarctic lakes, the distribution of microorganisms varies with lake type, physical and chemical environment, and local food webs, including inputs from terrestrial or marine vertebrate sources (Miller & Whyte, 2011). The mycota living in the Antarctic lakes is under the influence of various adverse factors, such as extremely low temperatures, frequent freeze-thaw cycles, high salinity, alkaline and acidic pH values, high UV radiation, and low nutrient availability (Gonçalves et al., 2012). Antarctic freshwater fungal communities typically include taxa of Ascomycota, Basidiomycota, Mortierellomycota, Mucoromycota, Chytridiomycota and allied species of Oomycota (Ogaki et al., 2019). Some fungal species occurring in Antarctic lakes are thought to be endemic, although the majority of these fungi are known as cosmopolitan cold-adapted species (Ogaki et al., 2019). However, although fungi are one of the dominant microbial groups present in Antarctic lakes and are assumed to play a fundamental role in the decomposition of suspended particulate organic matter in water from plants (degrading celluloses and lignocelluloses) and animals (exoskeletons, feathers, and hairs) (Wong et al., 1998; Martorell et al., 2019), detailed knowledge about the diversity and ecological roles of

fungi present in Antarctic lakes remain limited. Among the microorganisms reported in Antarctic lakes, fungi are known to be present at high diversity, although much of this currently remains undescribed (Ogaki et al., 2020 a, b). Early studies of fungal diversity in Antarctic lakes relied on traditional culture-dependent methods (Ellis-Evans, 1985, 1996; Gonçalves et al., 2012; Vaz et al., 2011; Ogaki et al., 2020a, b). However, in recent years, DNA metabarcoding approaches have started to be applied also in Antarctic lakes detecting the presence of sequences of different fungal taxa (Ogaki et al., 2021a; Rosa et al., 2022a; de Souza et al., 2022a, Gonçalves et al., 2022). Compared to Arctic region, more studies have reported fungi from different lakes and regions in Antarctica, including Deception Island (Gonçalves et al., 2012; Stanley & Rose, 1967; Vaz et al., 2011), King George Island (Vaz et al., 2011; Gonçalves et al., 2012, 2015; Ogaki et al., 2020a; de Souza et al., 2021), Signy Island (Willoughby, 1971; Ellis-Evans, 1985; McInnes, 2003), Vega Island (Ogaki et al. 2021a), Elephant Island (de Souza et al 2022a), James Ross Island (de Souza et al., 2022a), McMurdo Hope Bay (Antarctic Peninsula) (Rosa et al., 2022a), Dry Valleys (Goto et al., 1969; Knox & Paterson, 1973; Waguri et al., 1975; Waguri, 1976; Kriss et al., 1976; Nagashima et al., 1990; Baublis et al., 1991; de Hoog et al., 2005; Brunati et al., 2009; Connell et al., 2018; Rojas-Jimenez et al., 2017), Vestfold Hills (de Hoog et al., 2005; Brunati et al., 2009), Larsemann Hills (de Hoog et al., 2005; Brunati et al., 2009), Skarvsnes (Tsuji et al., 2013) and Vostok Station (D'Elia et al., 2009; Rogers et al., 2013). A list of the main research involving the fungal composition of lakes of different Antarctica regions is given in Table 1.

Table 1. List of fungal taxa from different substrata of Antarctica lakes in the literature from 1967 to date.

Region	Lake	Substrate	Fungal taxa	Reference
Maritime Antarctica				
Deception Island	Kroner, Relict, and two unnamed lakes Surface and depth water	Surface and depth water	*Rhodotorula*, *Candida*	Stanley & Rose (1967)
	Lake in Port Foster	Sediment	*Cystobasidium laryngis*	Vaz et al. (2011)
	Lake Crater	Water	*Pseudogymnoascus pannorum*, *Mortierella* sp., *Cladosporium cladosporioides*, *Penicillium* sp., *Davidiella tassiana*, *Cladosporium sp*., *Trichoderma longibrachiatum*	Gonçalves et al. (2012)
	Lake Crater Soto	Cellulose bait	*Tetracladium marchalianum*, *Tetracladium* sp., *Microsocial lycopodinum*, *Penicillium* sp., *Penicillium herquei*, *Leotiomycetes* sp., *Cladosporium* sp., *Mortierella gamsii*, *Mortierella parvispora*, *Chytridiomycota* sp., *Rozellomycota* sp.	de Souza et al. (2021)
		Biofilm	*Pseudogymnoascus* sp., *Penicillium* sp., *Mrakia blollopis*, *Vislmiacozyma victoriae*, *Rhodotorula mucilaginosa*, *Pseudogymnoascus verrucosus*, *Antarctomyces psychrotrophicus*, *Mortierella* sp., *Beauvearia amorpha*, *Artroderma* sp., *Holtermanniella wattica*, *Metsclmikowia australis*, *Debaryomyces* sp., *Tetracladium* sp., *Leucosporidium fragarium*	de Souza et al. (2022b)
		Sediment	*Talaromyces rubicundus*, *Aspergillaceae* sp., *Fusarium neocosmosporiellum*, *Dactylonectria anthuriicola*, *Betamyces* sp., *Chytridium* sp., *Goffeauzyma gastrica*	de Souza et al. (2022a)
King George Island	Lake in Agat point	Water	*Penicillium paneum*, *P. verrucosum*, *P. pannorum*, *Phaeosphaeria* sp., *Cadophora malorum*, *D. tassiana*, *Helotiales* sp., *Gibberella moniliformis*	Gonçalves et al. (2012)
	Lake next to the Brazilian Refuge II	Water	*Antarctomyces psychrotrophicus*, *Cladosporium cladosporioides*, *D. tassiana*, *Helgardia* sp., *P. pannorum*, *Microdochium* sp., *Microdochium nivale*, *Mortierella* sp., *Pleosporales* sp., *Saprolegniaceae* sp., *and Thelebolus* sp.	Gonçalves et al. (2012)
	Lake next to the Brazilian Station	Sediment	*Helotiales* sp., *Schizophyllum commune*	Gonçalves et al. (2015)
	Lake next to Copacabana USA Refuge	Freshwater and sediment	*Candida glaebosa*, *Nadsonia commutate* (freshwater); *Issatchenkia (Pichia) orientalis*, *Kodamaea ohmeri*, *Meyerozyma guilliermondii*, *Rhodotorula mucilaginosa*, *Vishniacozyma victoriae* (sediment)	Vaz et al. (2011)

	Lake in Jardew point	Sediment	*Annulohypoxylon* sp., *Cosmospora* sp.	Gonçalves et al. (2015)
	Two lakes next to Machu Picchu Station	Freshwater	*Aureobasidium pullulans*, *Exophiala xenobiotica, Leucosporidium creatinivorum, Microglossum* sp., *Rhodotorula mucilaginosa, Sporidiobolus salmonicolor, V. victoriae*	Vaz et al. (2011)
	One lake next to Machu Picchu Station	Freshwater	*Cladosporium* sp., *C. malorum, Fontanospora* sp., *P. pannorum, Helgardia* sp., *Mortierella* cf. *alpina, Phoma* cf. *paspali, Phaeosphaeria* sp., *Thelebolus s*p.	Gonçalves et al. (2012)
	Three lakes next to Machu Picchu Station	Sediment	*Aspergillus* sp., *C. malorum, Pseudogymnoascus* sp., *Penicillium* sp., *Pleosporaceae* sp., *Sordariomycetidae* sp.	Gonçalves et al. (2015)
	Lake next to Stain House glacier	Water	*A. psychrotrophicus, C. cladosporioides, Cosmospora* cf. *vilior, Cadophora* cf. *luteo-olivacea, P. pannorum, Helotiales* sp., *Heydenia* sp., *Microdochium* sp., *Mortierella* cf. *alpina, Phoma herbarum, Phoma fimeti, Thelebolus microspores*	Gonçalves et al. (2012)
	Lake next to Wanda glacier	Water	*Cladosporium* sp., *P. pannorum, Mortierella* sp., *Pseudeurotium* sp., *Phoma herbarum, Penicillium paneum, Thelebolus sp*	Gonçalves et al. (2012)
	North Lake	Sediment	*Cladosporium* sp., *Gyoerfyella* sp., *Helotiales* sp., *Glaciozyma* sp., *Phenoliferia psychrophila, Microbotryomycetes* sp., *Mrakia robertii, Neobulgaria* sp., *Penicillium glabrum, Phenoliferia glacialis, Phialophora alba, Pseudeurotium hygrophilum, Pseudogymnoascus* sp., *Pseudogymnoascus destructans, Pseudogymnoascus verrucosus, Vishniacozyma victoriae, Thelebolus globosus, Cystobasidium laryngis, Dioszegia fristingensis, Dioszegia hungarica, Kondoa changbaiensis, Kondoa malvinella, Kondoa subrosea, Hypocreales* sp., *Leucosporidium* sp., *Penicillium solitum, Pezizomycotina* sp., *Piskurozyma fldesensis, Sarocladium dejongiae, Sporobolomyces pararoseus, Vishniacozyma carnescens, Vishniacozyma victoriae*	Ogaki et al. (2020a)
	Central Lake	Sediment	*Cladosporium* sp., *Tetracladium globosum, Hypocreales* sp., *Neobulgaria* sp., *Penicillium* sp., *Pholiota baeosperma, Pseudeurotium hygrophilum, Pseudogymnoascus roseus, Pseudogymnoascus destructans, Antarctomyces pellizariae, Tetracladium globosum, Mortierella antarctica, Neobulgaria* sp., *Penicillium chrysogenum, Pseudeurotium hygrophilum, Pseudogymnoascus verrucosus*	Ogaki et al. (2020a)
	South Lake	Sediment	*Antarctomyces pellizariae, Cladosporium* sp., *Cystobasidium ongulense, Glaciozyma* sp., *Glaciozyma antarctica, Kriegeriales sp., Mrakia psychrophila, Penicillium glabrum, Penicillium solitum, Periconia byssoides, Polypaecilum* sp., *Pseudeurotium* sp.,	Ogaki et al. (2020a)

			Pseudeurotium hygrophilum, Pseudogymnoascus destructans, Pseudogymnoascus verrucosus, Thelebolus globosus, Glaciozyma martinii, Leucosporidium sp., *Patinella hyalophaea, Pseudogymnoascus roseus, Pseudogymnoascus verrucosus*	
King George Island	Lake Hennequin	Cellulose baits	*Tetracladium* sp., *Tetracladium marchalianum, Thelebolus globosus, Arthoniomycetes* sp., *Helotiales* sp., *Gyoerfyella entomobryoides, Alatospora acuminata, Knufa peltigerae, Chalara pseudoafnis, Hypocreales* sp., *Ramalinaceae* sp., *Microbotryomycetes* sp., *Mrakia frigida, Microbotryomycetes* sp., *Gofeauzyma* sp., *Glaciozyma antarctica, Pucciniomycetes* sp., *Mastigobasidium* sp., *Leucosporidiaceae* sp., *Mortierella* sp., *Chytridiomycota* sp., *Rozellomycota* sp., *Rhizophydium* sp., *Paranamyces uniporus, Rozellomycota* sp.	De Souza et al. (2022b)
Signy Island (South Orkney Islands)	Signy lakes	Freshwater	*Chytriomyces* sp., *Chytriomyces willoughbyi, Aphanomyces* sp.	Willoughby (1971)
		Freshwater	*Lagenidium giganteum, Hyphochytrium catenoides, Aphanomyces sp. (Saprolegniaceae), Rhodotorula* sp., *Leucosporidium* sp.	Ellis-Evans (1985)
		Benthic cyanobacterial mat and sediments	*Lecophagus antarcticus*	McInnes (2003)
Vega Island	Lake Pan Negro, Lake Esmeralda, Lake Copépodo	Sediment	*Pseudogymnoascus* sp., *Penicillium* sp., *Mortierella* sp., *Cladosporium* sp., *Pseudogymnoascus appendiculatus, Pseudogymnoascus roseus, Rozellomycota* sp., *Leotiomycetes* sp.,	Ogaki et al. (2021a)
Elephant Island	Lake Skua	Sediment	*Glaciozyma antarctica, Camptobasidiaceae* sp., *Mrakia* sp., *Mrakia psychrophile, Glaciozyma* sp., *Glaciozyma martini, Leucosporidilaes* sp., *Phenolipheria psychrophile, Tremellomycetes* sp., *Mrakia niccombsii, Monoblepharidales* sp., *Pseuderotium* sp., *Ciliophora* sp., *Pseudogymnoascus* sp., *Naoascochyta paspali, Penicillium* sp., *Antartomyces* sp., *Penicillium nalgiovense, Aspergillaceae* sp.	de Souza et al. (2022a)
James Ross Island	Lake Florencia	Sediment	*Aspergillaceae* sp., *Penicillium nalgiovense, Pseudogymnoascus* sp., *Ciliophora* sp., *Pseudeurotium* sp., *Scutellinia* sp., *Acremonium biseptum, Clathrosphaerina zalewskii, Saccharomycetales* sp., *Thelebolus balaustiformis, Cladosporium* sp., *Helotiales* sp., *Lobulomycetales* sp., *Rhizophydiales* sp., *Chytridiales* sp., *Glaciozyma antarctica, Camptobasidiaceae* sp., *Rozellomycotina* sp., *Sanchytrium* sp.	de Souza et al. (2022a)

	Lake Katerina	Sediment	*Talaromyces rubicundus, Tetracladium* sp., *Sordariales* sp., *Spizellomycetales* sp., *Lobulomycetales* sp., *Betamyces* sp., *Agaricales* sp., *Rozellomycotina* sp., *Mortierellales* sp.	de Souza et al. (2022a)
	Lake Lilia, Lake Cecilia, Lake Soledad and Lake Adriana	Sediment	*Betamyces* sp., *Rozellomycotina* sp., *Talaromyces rubicundus, Neoascochyta paspali, Ciliophora* sp., *Penicillium nalgiovese, Pseudogymnoascus* sp., *Rhizophydiales* sp., *Chytridium* sp., *Cgytridiales* sp., *Candida parapsilosis, Fusarium neocosmosporiellum, Cladosporium* sp.	Gonçalves et al. (2022)
Hope Bay	Lake Boeckella	Sediment	*Pseudeurotium hygrophilum, Rozellomycota* sp., *Pseudeurotiaceae* sp., *Chytridiomycota* sp., *Microbotryomycetes* sp., *Wallemia* sp., *Tetracladium* sp., *Debaryomyces* sp., *Chytridiomycota* sp., *Malassezia globosa, Nectriaceae* sp., *Xylaria apiculata, Pseudeurotiaceae* sp., *Microascales* sp., *Aspergillus flavus, Cystobasidium pinicola, Glaciozyma* sp., *Naganishia* sp., *Mortierella* sp., *Pseudogymnoascus* sp., *Celeophoma* sp., *Curvularia* sp., *Hyphodiscus* sp., *Cladosporium* sp., *Penicillium* sp.	Rosa et al. (2022a)
Ice-free areas (continental Antarctica)				
McMurdo Dry Valleys (Southern Victoria Land)	McMurdo oasis next to McMurdo Station	Freshwater, soil, and algae	*Scherffelliomyces appendiculatus, Chytridium versatile, Rhizophlyctis rosea, Rhizophydium proliferum, Phlyctochytrium recurvastomum, Catenophlyctis variabilis, Aphanomyces (Saprolegniales), Pythium tenue, and Pythium sp.*	Knox & Paterson (1973)
	Lake Bonney	Soil in lake side and inflow stream	*Candida australis*	Goto et al. (1969)
		Bottom water	*Dendryphiella* sp., *Diheterospora catenulata*	Waguri et al. (1975), Waguri (1976)
	Lake Basins located in the Taylor and Miers Valleys (including two samples from Lake Bonney)	Water	*Cryptomycota* sp., *Chytridiomycota* sp., *Ascomycota* sp., *Zygomycota* sp., *Blastocladiomycota* sp., *Glaciozyma* sp., *Mrakia* sp.	Rojas-Jimenez et al. (2017)
	Lake Fryxell	Algae in lake side	*Candida scottii*	Goto et al. (1969)
		Bottom water	*Aureobasidium foliicolum*	Waguri et al. (1975), Waguri (1976)

		Biomats	*Thelebolus ellipsoideus, Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Embellisia* sp., *Onychophora* sp., *Glaciozyma antarctica, Mrakia frigida*	Brunati et al. (2009)
		Water	*Acremonium* sp., *Aureobasidium pullulans, Cladosporium cladosporoides, Clavispora lusitaniae, Debaryomyces hansenii, Pseudogymnoascus* sp., *Heydenia alpina, Penicillium commune, Penicillium dipodomyicola, Thelebolus ellipsoideus, Thelebolus globosus, Toxicocladosporium strelitziae, Filobasidium magnus, Glaciozyma antarctica, Glaciozyma watsonii, Holtermanniella yarrowii, Mrakiella aquatica, Naganishia albidosimilis, Naganishia globosa, Rhodotorula mucilaginosa, Vishniacozyma victoriae*	Conell et al. (2018)
	Lake Hoare	Meltwater foam, microbial mat from benthos and from meltwater	*Candida ciferrii, Cephalosporium acremonium, Aureohasidium pullulans, Chrysosporium pannorum, Geotrichum candidum, Penicillium notatum*	Baublis et al. (1991)
		Biomats	*Thelebolus ellipsoideus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Leucosporidium antarcticum, Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Lake Miers	Water and an outlet stream	*Candida diffluens, R. texensis* (in water)*, Rhodotorula rubra var. miersensis* (in an outlet stream)	Goto et al. (1969)
	Lake Vanda	Water and sediment	*Cryptococcus albidus, Candida diffluens, Candida humicola, Trichosporon cutaneum var. antarcticum, Rhodotorula glutinis var. rufusa, Rhodotorula texensis* (water)*; Sporobolomyces antarcticus, C. diffluens, C. scottii, Rhodotorula rubra* (sediment)	Goto et al. (1969)
		Deep water (68 m)	*Aspergillus, Penicillium, Stachybotrys, Trichoderma*	Kriss et al. (1976)
		Water from several depths (5–69 m)	*Candida* sp.	Nagashima et al. (1990)
Vestfold Hills	Lakes Ace	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp., *Cladosporium* sp., *Pseudogymnoascus* sp.*; Cryptococcus albidus, Cryptococcus infirmominiatus, Cryptococcus laurentii, Leucosporidium*	Brunati et al. (2009)

			antarcticum, Leucosporidium scottii, Mrakia frigida, Rhodotorula mucilaginosa	
	Lake Druzby	Biomats	*Thelebolus ellipsoideus, Thelebolus globosus, Thelebolus* sp.	de Hoog et al. 2005
		Biomats	*Thelebolus sp., Phoma sp., Leucosporidium scottii*	Brunati et al. (2009)
	Lakes Highway	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp.	Brunati et al. (2009)
	Lake Organic	Biomats	*Thelebolus microsporus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp.	Brunati et al. (2009)
	Lake Pendant	Biomats	*Acremonium* sp., *Alternaria* sp., *Arthrinium* sp., *Aspergillus* sp., *Beauveria* sp., *Botrytis* sp., *Penicillium* sp., *Cladosporium* sp., *Curvularia* sp., *Pseudogymnoascus* sp.	Brunati et al. (2009)
	Lake Watts	Biomats	*Thelebolus globosus*	de Hoog et al. 2005
		Biomats	*Thelebolus* sp., *Penicillium* sp., *Beauveria* sp., *Phialophora* sp.	Brunati et al. (2009)
Larsemann Hills (Princess Elizabeth)	Lake Manning	Biomats	*Thelebolus ellipsoideus, Thelebolus microsporus, Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., *Cladosporium* sp., *Curvularia* sp., *Rhodotorula minuta*	Brunati et al. (2009)
	Lake Reid	Biomats	*Thelebolus microsporus*	de Hoog et al. (2005)
		Biomats	*Thelebolus* sp., *Phoma* sp., *Cladosporium* sp., *Candida lipolytica, Cryptococcus albidus, Debaryomyces hansenii var. hansenii, Leucos poridium scottii, Rhodotorula mucilaginosa*	Brunati et al. (2009)
	Lake Sarah Tarn	Biomats	*Thelebolus* sp.	de Hoog et al. (2005)
		Biomats	*Aspergillus* sp.	Brunati et al. (2009)
Skarvsnes (Lutzow-Holm Bay, East Antarctica)	Abi-ike, Ageha-ike, Bosatsu-ike, Ebi-numa, Hyoutan-ike, Jizou-ike, Kuwai-ike, Kumogata-ike, Magoike,	Surface soil around lakes and sediments	*Embellisia* sp., *Phoma* sp., *Pseudogymnoascus* sp., *Tetracladium* sp., *Thelebolus* sp., *Mrakia* sp., *Cryptococcus* sp., *Dioszegia* sp., *Rhodotorula gracialis, Leucosporidium antarcticum*	Tsuji et al. (2013)

	Naga-ike, Nisehyoutan-ike, Nyoraiike, Ohgi-ike, Oyako-ike, Shimai-ike, and Tokkuri-ike lakes			
Subglacial lakes				
Vostok Station (under surface of the central East Antarctic ice sheet)	Lake Vostok	Accretion ice	*Cystofilobasidium* sp., *Cryptococcus* sp., *Pseudozyma* sp., *Penicillium* sp., *Aeurobasidium* sp., *Aspergillus* sp.	D'Elia et al. (2009)
		Accretion ice and surface of the southern main basin	*Ascomycota, Basidiomycota, Zygomycota*	Rogers et al. (2013)

Thanks to recent DNA metabarcoding, other than the commonly encountered phyla Ascomycota, Basidiomycota, Mortierellomycota, Mucoromycota, Chytridiomycota, Rozellomycota and Zoopagomycota, sequences belonging to the more rarely detected or unreported Aphelidiomycota, Basidiobolomycota, Blastocladiomycota and Monoblepharomycota, as well as members of the fungal-like kingdom Straminopila were also detected (de Souza et al., 2022a; Gonçalves et al. 2022). These studies revealed that the fungal sequence diversity present in Antarctic lakes is large and complex, suggesting that Antarctic lakes may represent a hotspot of fungal diversity in Antarctica.

2.5 CONTAMINATION OF POLAR LAKES BY HEAVY METALS

2.5.1 How Heavy Metals reach Polar regions?

The widespread perception is that than Arctic region, being situated far from all major cities and manmade 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 long as these regions are not free of pollution. In fact, the presence of contaminants that originate from local and global sources is well documented. They include organic as well as inorganic compounds (Szopinska et al., 2017). The organic contaminants include persistent organic pollutants (POPs) and pesticides. Inorganic contaminants consist predominantly of heavy metals (HMs). Because of the high solubility of HMs in the aquatic medium, they can be easily absorbed or taken by the organisms. On the other hand, being metal, they are non-biodegradable and have multiple threats to microorganisms, plants, animals, and humans due to their persistence in nature, high-toxicity characteristics, and long half-lives after coming into the food chain (Ali et al., 2015; Jamali et al., 2007; Mondal et al., 2017). 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.

Heavy metals have a natural origin, being substantially a natural component of soils, in which they can exist in organic fraction as a result of degradation or organic metal or as mobile metal species or coming from other natural sources such wind-blown crustal dust, sea-spray, and volcanism (Dick, 1991). For instance, volcanic activity is an important Hg source in Deception Island, where Hg levels in water and sediments sampled at two fumaroles were up to 10.000 times higher than in the other sampling sites (Mao de Ferro et al., 2014). In Polar regions, animals represent another natural source of heavy metal contamination, in fact animal-impacted sites are often areas with naturally elevated levels of heavy metals. For instance, Evans et al. (2017) recently reported that orinthogenic 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 biotransporting 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 the Antarctic (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 and Sun, 2007). Animal carcasses are another important natural source of heavy metal contamination. For instance, seal carcasses have been shown to be a significant source of Hg and methylmercury contamination in Antarctic soils (Zverina et al., 2017). 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 scale (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 et al., 2008; Kosek & Ruman, 2021) 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 in areas affected by scattered rubbish or emissions from incinerators, generators and vehicles (Kennicutt et al., 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 into 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 to global circulation and reach the Arctic region and the Antarctic continent via long-range oceanic and atmospheric transport (Truzzi et al., 2017; Zabaroska et al., 2017; Chu et al., 2019; Rajaram et al., 2023). 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) (Rudels et al., 1999; 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 a number of 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). Global climate change has been found to play an important role in contaminant discharge in Polar regions. In Arctic, for example, glaciers have accumulated contaminants for decades and now, due to increases in glacier melting, can introduce large amounts of those compounds to fjord waters (Macdonald et al., 2005; Zaborska et al., 2017).

2.5.2 Polar lakes contamination

Due to extreme environmental conditions such as low temperature, strong radiations, mostly low buffering capacity and low nutrient level, lake ecosystems in Polar regions have a relatively simple food web and react more rapidly and more sensitively to environmental changes than other lakes (Bharti et al., 2013). For these reasons, polar lakes are sensitive reference systems of human impacts (Bharthi et al., 2012). These lakes have been increasingly affected by anthropogenic activities such as various research activities, tourism, mining, and logistic support, or for those lakes that are found in remote locations in general protected from direct human impacts, are being polluted due to airborne contaminants, including heavy metals (Bhardwaj et al., 2023). The anthropogenic impact on Arctic lake was evaluate in a very recent study by Rajaman et al. (2023). They determined the concentrations of five heavy metals (Cd, Cr, Cu, Pb, and Zn) from different abiotic and biotic samples from Ny-Ålesund in the Svalbard archipelago, including four freshwater lakes (Tvillingvatnet, Knudsenheia, Storvatnet, and Solvatnet). Among the polluted sites, freshwater lake Solvatnet, located very near to the town of Ny-Ålesund, resulted as the most heavily polluted area. The detection of elevated levels of heavy metals could be attributed to anthropogenic activities from the port and human settlements nearby (Rajamar et al., 2023). The impact of coal mining activity and coal combustion in Longyearbyen on Svalbard Island on local lakes was reported by Sun et al.

(2018). In particular, in a 150-year record of heavy metals (Pb, As, Cd, Cu, Cr, Co, Ni, and Sn) in the sediment of Lake Bolterskardet, it was observed that heavy metal fluxes, in particular for Pb, increased during the period of major activity of coal mines and with an increase of coal combustion in the Longyearbyen power station. Increasing heavy metal content such as Ni, Cu, Pb, Cd, and Hg in small Arctic mountain lakes, was also associated to anthropogenic impact (atmospheric emissions from the adjacent mines) and transboundary transport of contaminants in the high atmosphere layers (Dauvalter et al., 2022). Antarctic pollution research has been historically focused on sea water and snow or ice (Abouchami et al., 2011; Corami et al., 2005; Stedmon et al., 2011; Toyota et al., 2011), but an underestimated quantity of useful information can be gained studying lacustrine systems. In fact, lake waters and sediments are the main sinks for solutes and particulate materials thus making lacustrine ecosystems a short- and long-term catchers of biogeochemical processes (Borghini & Bargagli, 2004). Contamination by heavy metals due to anthropogenic input was reported also for Antarctic lakes, in particular in the vicinity of the research stations (Burgess & Kaup, 1997; Gasparon & Burgess 2000; Goldsworthy et al., 2003). Several studies reported the high concentration of heavy metals such as Pb, Cu, Zn, and Hg in lakes located in both maritime (Nedzarek et al., 2014; Bueno et al., 2018) and continental Antarctica (Singh & Tiwari, et al., 2008), stating that the presence of heavy metals in the lakes may be due to the proximity of research stations. Nevertheless, in some cases, the contribution of human activities to total concentrations of trace metals in water and sediments is negligible. For instance, Gasparon & Matschullat (2006) studied the heavy metals in the sediment samples collected from Progress II, Lake Heart, Zhong Shan Tarn, Lake Bruehwiler, and Lake Nella from Larsemann Hills, East Antarctica. They reported Pb and Zn within the below detection limits. Compared results were obtained for another lake in Larsemann Hills (Fisher Island), where low concentrations of metals Cu, Pb, Cd, Zn and Cr showed that the lake water of the lake has no pollution load and no impact of any anthropogenic activity (Barthi et al., 2015). In a study by Abollino et al. (2004) the concentrations of trace

elements, in particular those of heavy metals in five shallow Antarctic lakes found in Terra Nova Bay, resulted very low, suggesting an origin from natural sources (sea-spray input and some extent by shallow-level groundwater and surface water input and, hence, by rock- and sediment-water interaction processes) rather than from anthropogenic contamination research stations or from global air circulation. In the other four small lakes investigated in Terranova Bay, a strong correlation between elements considered potential anthropogenic pollutants (e.g., As, Pb, Zn, Cu and Ni) with the lithogenic elements (e.g. Al, Si, Fe) suggests that their origin is connected to natural phenomena (Zelano et al., 2017), as suggested by previous research.

2.6 HEAVY METAL TOLERANCE AND REMOVAL MECHANISMS IN FUNGI

Metals play critical roles in fungal homeostasis. They are required for various biochemical processes, usually as enzymatic cofactors. For example, In the model yeast *S. cerevisiae* it was observed that zinc plays a crucial role as large portions of zinc-binding proteins are related to critical functions, including DNA binding, the regulation of transcription, transcription factor activity, and response to chemical stimuli (Wilson et al., 2012; Staats et al., 2013). Copper is necessary for the activation of metalloproteins involved in the activation of superoxide dismutase, which is responsible for the cellular detoxification of reactive oxygen species (ROS), virulence in pathogenic species, and activation of cytochrome c oxidate, a catalyst within the electron transport chain (Smith et al., 2017; Raffa et al., 2018). Iron is also essential for fungal virulence in pathogenic species, most importantly as an integral component of iron-sulfur clusters which are required for the activation of nuclear proteins involved in DNA repair (Lindahl et al., 2019). Copper and manganese also play a critical role in fungi, in particular, in filamentous species where it is required for the activation of manganese peroxidase, which could be used as a secondary metabolite to depolymerize lignin for nutrients (Janusz et al.,

2013). Fungi have been reported to exhibit significant tolerance/resistance towards HMs and turn out to be dominant organisms in some contaminated aquatic water bodies and other habitats (Gola et al. 2016; Kumar and Dwivedi 2019a; Mishra and Malik 2012). For this reason, fungi have great potential for their application in HM remediation. In case of excessive metal exposure, fungi could activate some relevant resistance mechanisms. There are some mechanisms in fungi to tolerate and detoxify the HMs: enzymatic detoxification, accumulation inside the cell via active (transport systems) and/or passive (diffusion) uptake mechanism, exclusion by permeability barrier, adsorption on extracellular structures (cell wall, capsule, slime), extra- and intracellular precipitation, efflux pumps, the adjustment in the cellular targets, methylation, volatilization and chelation of metal/loids, etc. (Baldrian 2003; Gadd 2007; Merroun et al. 2001; Zhang et al. 2005). For example, it was observed that in *Saccharomyces cerevisiae*, short-term exposure to $CuSO_4$ results in the upregulation of *CUP1* and *CRS5* (metallothioneins binding copper) and the downregulation of *FRE1* (ferric/cupric reductases which reduce siderophore-bound iron and oxidized copper prior to uptake by transporters) and *CTR1* (high-affinity copper transporters of the plasma membrane which mediate nearly all copper uptake) (Shi et al., 2021). In *Aspergillus* spp., P-type ATPase CrpA has Cu exporting activity that aids in cellular detoxification, increasing Cu resistance (Antsotegi-Uskola et al., 2020). In *Fusarium graminearum*, copper exposure upregulates FgCrpA (ATPase exporter) and the MT FgCrdA as a means to prevent over accumulation (Liu et al., 2020). In *F. oxysporum*, upregulation of oxidoreductase activity may decrease susceptibility to oxidative stress that can be induced by excessive copper exposure (Ragasa et al., 2021). Iron is involved in many biological processes but can be toxic in excess. Iron resistance in the species *S. cerevisiae* showed that the fungus achieves iron resistance through the downregulation of iron import systems, or by the activation of vacuolar transporter Ccc1 (Berthelet et al., 2010) and by the expression of ferritin related genes (Shin et al., 2021).

The negatively charged cell wall surface plays a crucial role in the adsorption of positively charged metal ions via electrostatic attraction (Kumar & Dwivedi, 2019a). The fungal cell wall, in fact, is mostly made up of protein, polysaccharides, polyphosphates, polypeptides, lipids, chitin, inorganic ions, etc. (Ayangbenro & Babalola, 2017; Kang et al., 2018) which contain a number of functional groups such as – COOH, –OH, –NH2, =NH, –SH, –O–CH3, etc. which is mainly responsible for the bioadsorption of HMs on the surface of fungal cell. Copper ion adsorption with the amine, carboxyl, and phosphate groups was reported by Majumdar et al. (2008). After adherence to the surface, fungi accumulate the metal from outside to inside the cell via active or passive mechanisms. In passive mechanism, the metal may come inside the cell via ion exchange or osmotic mechanism, while in the case of active transport, it is driven by many types of transporting agents which are involved in the transport of the metal from the cytoplasm to the periplasm or vacuoles of the cell. In fungi, many types of transporters have been reported for the transport of the HMs from outside to inside of the cell or from periplasm to cytoplasm or from cytoplasm to vacuoles or from inside to outside of the cell (in case of efflux pump), such as Cd-conjugated ATP-binding cassette (ABC) transporter, manganese transporter, iron transporter, copper transporter and metal transporting ATPase (Bellion et al., 2006). In case of HM stress conditions, ROS may work as signaling molecules for the production of glutathione, metallothionein, phytochelatins, and other thiolic compounds that work as metal-chelating agents and transport them into vacuoles or outside of the cell through an efflux pump (Chen et al., 2019; Singh et al., 2016; Kumar & Dwivedi, 2019a, b; Banerjee et al., 2019). Some chelating agents such as metallothionein (Vaseem et al., 2017) and phytochelatins (Jacob et al., 2016) are present inside the fungal cells and play an important role in the detoxification of HMs. These chelating agents form complex compounds by binding with the metal ion which is usually secreted by fungi as secondary metabolites or can be accumulated inside the vacuoles of the cell (Vaseem et al., 2017). Melanin, a dark pigment produced by some fungal species, has various functional groups (phenolic, carboxyl, alcoholic hydroxyl, carbonyl,

methoxy groups, etc.) which are mainly responsible for the chelating potential of fungi (Butler & Day 1998; Pombeiro-Sponchiado et al., 2017). Siegel (1987) reported that the melanin rich fungal strain *Cladosporium cladosporioides* has more potential to biosorb Cd, Ni, Cu, Zn, and Pb compared with non-melanized *Penicillium digitatum*. Bioprecipitation is another of the main mechanisms which is involved in the removal of HMs by microbes (Maisa et al., 2018) The main anionic species involved in bioprecipitation are PO_4^{2-}, CO_3^{2-}, S^{2-}, OH^-, $C_2O_4^{2-}$, O_2─, Cl─, etc. In intra cellular precipitation, metal ion comes inside the cell through the fungal cell wall and precipitate (sable/insoluble compound) as their minerals by reacting with respective anionic species, while in extracellular precipitation, metal ion, extracellularly synthesized into their precipitate where anionic species may be donated by the fungus or may be provided from their surrounding medium, while the fungal cell surface provides a base for the reaction to take place. For instance, Liang et al. (2015) reported phosphate, sulfate, oxide anion involvement in the removal of lead and found lead phosphate ($Pb_3(PO_4)_2$), anglesite ($PbSO_4$), and pyromorphite ($Pb_5(PO_4)_3Cl$), the lead oxides massicot and litharge (PbO) as a lead precipitate.

Biovolatilization basically deals with the biological volatilization of metal from the water and soil into the environment. Hg which is one of the toxic metals and is volatile and can also be biovolatilized by microorganisms. Bacterial as well as fungal volatilization of Hg is frequently reported in many studies that play an important role in the decontamination of the Hg-polluted site (Urík et al., 2014; Wang et al., 2018; Chang et al., 2020). Generally, bacteria and archaea utilize the *mer* operon which is capable of enzymatic reduction of Hg(II) or methyl mercury (MeHg) to less toxic Hg(0), volatile species of Hg (Boyd & Barkay 2012; Giovanella et al., 2016). In fungi, the mechanism of biovolatilization of Hg is not well characterized. However, in a recent report, it is found that *mer* genes (merA) are upregulated in the exposure of Hg(II) in Penicillium spp. (Chang et al., 2020). They have also analyzed the activity of mercuric reductase that is responsible for the reduction of Hg(II) to Hg(0). Thus, the *mer* operon is basically involved in enzymatic reduction of Hg(II) to Hg(0) as well as its volatilization. Some

other fungal species such as *Candida albicans*, *Saccharomyces cerevisiae* (Yannai et al., 1991), *Scopulariopsis brevicaulis* (Boriová et al., 2014), *Aspergillus niger*, and *Cladosporium* sp. (Urík et al. 2014) also have been reported for volatilization of Hg, but no other clear Hg volatilization pathway has yet been observed in fungi. However, in live fungi, the optimum temperature for the growth of fungi usually varies between 25–35 °C, and the removal rate basically depends on the growth of the fungi (Prasad et al., 2018; Kumar & Dwivedi, 2019a) In cold environments, biodegradation of organic pollutants and heavy metal bioconversion requires the activity of cold-adapted or cold-tolerant microorganisms. The need for HM resistant fungi autochthonous to a contaminated site has been recently emphasized for in situ bioremediation approaches (Kan et al., 2019; Chang et al., 2020; Gururajan et al., 2020; Oladipo et al., 2018). The advantages are obvious: these isolates are already adapted to site-specific conditions, especially to competition within local communities, and are equipped with resistance/tolerance mechanisms that may account for toxicity reduction or removal of HMs at the site.

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3 AIM OF THE WORK

The aim of this thesis was to study the fungal community of Arctic and Antarctic lakes and its ecological function, with particular attention to the isolation of heavy metal tolerant micro-fungi. The analyses were conducted on water and sediments lakes samples collected at the Svalbard area and at the South Shetlands Archipelago. These sites were chosen because lake ecosystems, especially small lakes, and ponds, are widely distributed in these areas. Furthermore, lakes are very sensitive to environmental perturbations, including pollutants such as heavy metals.

The tasks that were envisaged to reach the main objective were:

I. Analysis of the fungal community composition by cultural-dependent and cultural-independent methods.

II. Enrichment, isolation and identification of microfungi which were able to tolerate high concentrations of heavy metals.

III. Study of the ecological function of the fungal community.

4 MATERIALS AND METHODS

4.1 SAMPLING AREAS AND SAMPLES DESCRIPTION

Two sampling campaigns were carried out in Arctic and Antarctic areas, in which 5 Artic lakes and 7 Antarctic lakes were sampled. The sampling campaigns were conducted in the frame of MicroPolArS "Microbial response to human Pollutants in polAr lakeS" project (PNRA 18_00194) as described below.

4.1.1 Lakes in Ny-Ålesund (Svalbard Islands, High Artic)

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 Svalbard Islands. Ny-Ålesund is warmer and more humid than elsewhere with similar latitude in the Northern hemisphere, due to the changes in atmospheric circulation and 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 typical High Arctic ecosystems of Svalbard, and most of the islands' animal and plant species can be found in this area, making the city an ideal starting point for conducting research on the flora and fauna of the Arctic Circle. Ny-Ålesund is a former Norwegian coal mining town and was closed in the 1960s due to a tragic accident (Hisdal et al., 1998). At present, there are several tens of all-year permanent residents (scientific expeditioners and logistical support personnel), and the population reaches more than one hundred in summer. Because of its unique climate qualities 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 lakes surrounding the Ny-Ålesund research town. Five

different lakes were selected for sampling which includes Solvannet (L1), Glacier (L2), Knudsenheia (L3), Storvatnet (L4) and Tvillingvatnet (L5) (Figure 1).

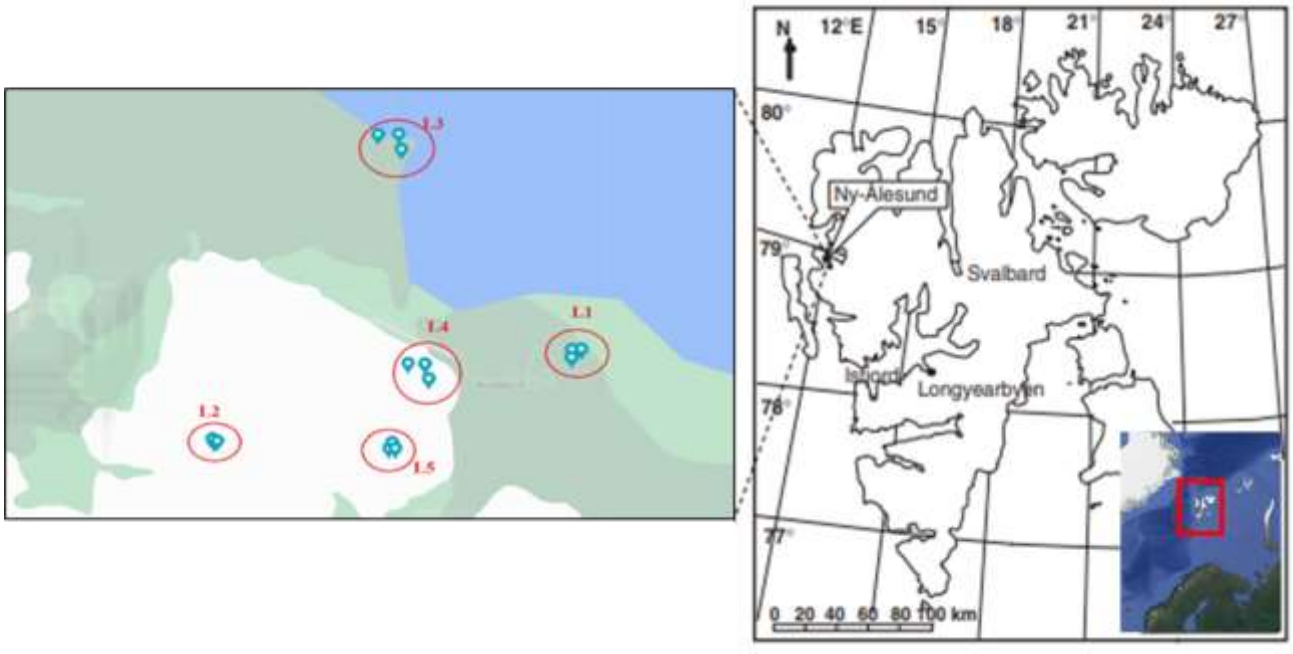


Figure 1. Maps showing Svalbard Islands and lakes sampled in the Ny-Ålesund area.

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 physical-chemical data for each sampling point are shown in Table 2.

Table 2. Geographical and physical-chemical data for each sampling site.

Area	Lake	Sample ID	Coordinates		Physio-chemical parameters			
					Water temperature (°C)	pH	O2 %	Cond (uS/cm)
Ny-Ålesund	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	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	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

4.1.2 Lakes in Deception Island and Livingston Island (Antartica)

Deception Island (62°57'S, 60°38'W), is located in the South Shetland Islands archipelago, lies in 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 in maximum diameter, rising to 539 m at Mount Pond, and 452 m at Mount Kirkwood, both summits being covered by permanent ice. About 57% of the island is covered by ice caps, glaciers and ice-cored 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). Livingston Island has a maritime climate, less extreme than on the Antarctic Continent with temperatures comprised 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 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) (Figure 2). Geographical coordinates and physical-chemical data for each sampling point are shown in Table 2.

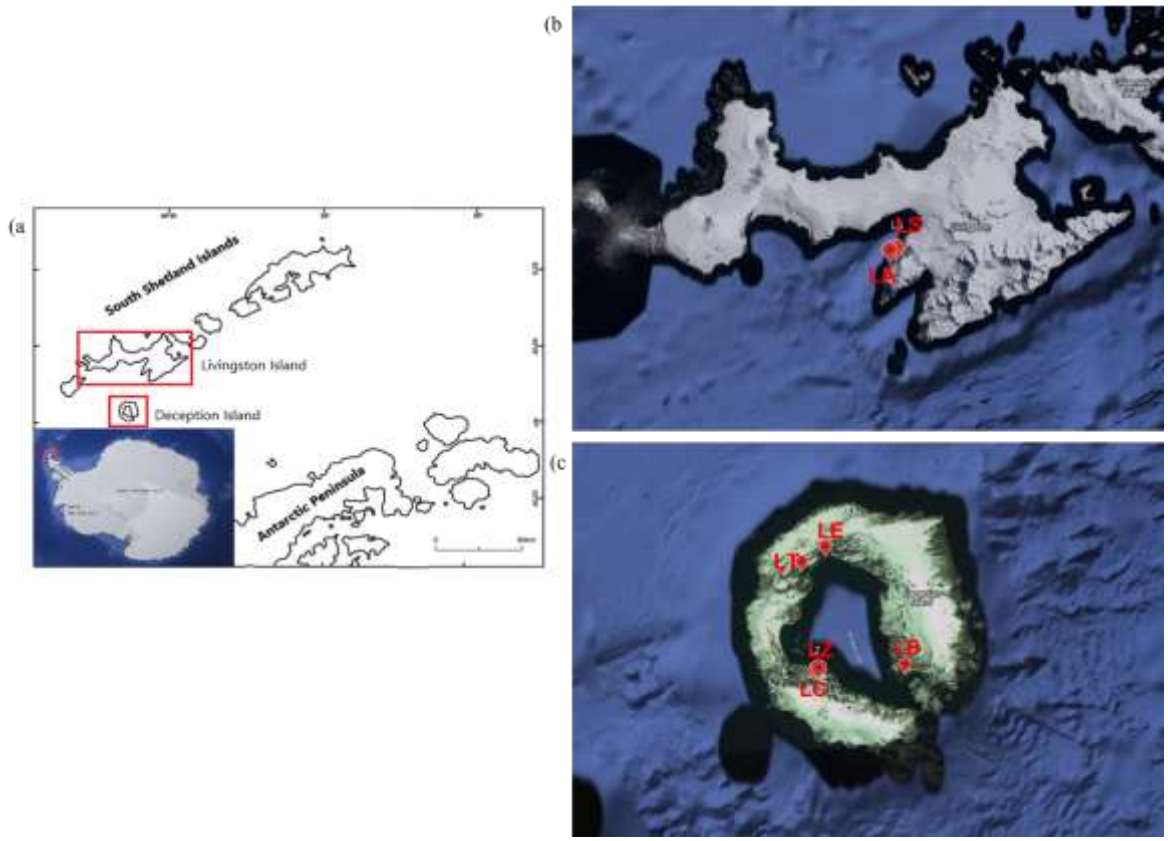


Figure 2. Maps showing a) Deception and Livingston Islands and in detail, the locations of lakes sampled in b) Livingstone Island and c) Deception Island.

4.1.3 Collection of samples

For each lake, water and sediment samples were collected from three almost equidistant points, except for Crater, Extremadura and Zapatilla lakes in Antarctica from which one or two ssampling points were considered due to difficulties in sampling (Table 2).

Water samples were manually collected from the littoral zone of each lake using a pre-sterilized 2 L plastic bottle and immediately transported to the labs of the research stations to be processed. For cultural analyses 5 mL, 50 mL, 100 mL and 200 mL aliquots of water samples were filtered through membrane filters 0.45 μm pore size with 47 mm diameter (Millipore), in duplicate. Filters were immediately stored in 15 mL sterile tubes containing a solution of glycerol 50% final concentration and stored at – 20 ° C for transport in Messina Labs.For the molecular study of fungal community, 1 L of water samples was filtered on polycarbonate membranes (diameter 47 mm; 0.22 μm pore size), in triplicate, and immediately frozen at −20 °C for transport in Italy.

Sediment samples were manually collected from the littoral zone of each lake at a depth of 30–60 cm. The first 10 cm of the superface sediment were sampled using a pre-cleaned scoop and pre-sterilized plastic containers and immediately transported to the labs of the research stations. For cultural analysis, 1 g sub-sample was transferred in 10 mL of filter sterilized water coming from the same sampling site and glycerol (50% final concentration) and stored at -20 °C. For the molecular study of fungal community, sediment samples were directly stored at – 20 °C to be processed in Messina labs.

Temperature, pH, dissolved oxygen, conductibility, and temperature of water were recorded at each sampling point (Table 2).

4.2 STUDY OF FUNGAL COMMUNITY DIVERSITY

The study of fungal community diversity was carried out by a multi-step approach, using traditional culture-dependent method by the isolation of microfungi on culture media, and by culture-independent methods i.e. metagenomics, which allows access to a microbial genetic pool that is not reachable through classical microbial cultivation techniques.

4.2.1 Cultural-dependent analysis

4.2.1.1 Fungal isolation from water and sediment samples

For the isolation of fungal strains from water samples, membrane filters obtained as described above, were placed on two cultivation media: Dichloran Rose Bengal Chloramphenicol (DRBC, Condalab, Spain), a general-purpose enumeration medium (King et al., 1979), and YM agar (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, 2% dextrose, 2% agar) containing 100 mg/mL of chloramphenicol (Sigma) (Gonçalvez et al., 2012) and incubated at 4°C up to 30 days.

To isolate the fungal species from sediment samples, the 1 g of subsample suspended in 9 mL of sterile lake water and glycerol, prepared as described below, was vortexed and serial dilutions up to 10^{-3} in saline solution 0,85% were prepared. Then, 100 µL of each dilution were spread plated, in double, onto DRBC agar medium and YM agar medium containing 100 mg/mL of chloramphenicol and incubated at 4 °C for 30 days.

Fungal colony-forming units (CFUs) were counted and subcultured in YM. The subcultures were grouped into different morphotype according to their cultural (colony colour and texture, border type and radial growth rate) characteristics on YM agar. All fungal isolates obtained were deposited in the Collection of Department of Chemical, Biological, Pharmaceutical and Environmental Sciences of the University of Messina, Italy.

4.2.1.2 Isolation of HM tolerant fungal strains from enrichment cultures

To set up culture enrichment from each sediment samples, 1 g of wet sediment was used 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), with a final concentration of 1000 ppm for Fe and Cu, and 100 ppm of Hg. All enrichments were incubated aerobically at 4 °C with shaking at 175 rpm for 1 month (Rappazzo et al., 2019).

After incubation, aliquots (100 µL) of each enrichment were spread plated, in double, on YM agar 10% containing chloramphenicol 100 mg/mL and the respective HMs used for the enrichments (Fe, Cu e Hg), separately, with the same concentrations (1000 ppm for Fe and Cu, and 100 ppm for Hg). The culture medium YM agar 10% without metals was used as negative control. Replicate plates were incubated at 4 °C for 30 days. For fungal isolation, 5-6 colonies were randomly selected from agar plates, picked, and subcultured three times on YM 10 % agar medium.

4.2.1.3 Test of growth with increasing concentrations of HMs

Each fungal isolate was then tested for growth at increasing concentrations (up to 5000 ppm for Fe and Cu, and up to 500 ppm for Hg) of the same HM amending the isolation medium. For this, we set up a growth test in Microtiter plates and measured the optical density of the fungal cultures over time, using a modification of the protocol described by Blasi et al. (2016).

Starting cultures were prepared by cultivating each fungal strain on the Microtiter plate (Labsolute), in 250 µL of YM 10% medium, in duplicate. Plates were then incubated at 4 °C for 15 days. Growth was evaluated daily by measuring optical density using a microplate reader Adsorbance 96 spectrophotometer (Byonoy, Germany) at $\lambda = 605$ nm and values were recorded by the software Absorbance 96. Wells containing YM 10% medium without fungal inoculation

were used as blank for the OD readings. To calculate the change in OD 605, the blanks were subtracted from the OD 605 values of all tested strains. After incubation, 25 µL of each fungal culture were cultivated in duplicate in 225 µL YM 10 % containing the same HM amending the isolation medium, using the following concentrations: 2500 ppm and 5000 ppm for Fe and Cu, and 250 ppm and 500 ppm for Hg. Plates were then incubated at 4 °C for 30 days. Growth was evaluated daily as described below. Well containing YM 10% medium plus HM without fungal inoculation were used as blank for the OD readings.

4.2.1.4 Screening for HM multi-tolerance

Fungal strains which could grow in the presence of higher concentrations of HMs were further assayed for tolerance to heavy metals different from that used for the enrichment cultures.

As previously described, selected strains cultures were prepared by cultivating each fungal strain on the Microtiter plate, in 250 µL of YM 10% medium, in duplicate. Plates were then incubated at 4 °C for 15 days. Growth was evaluated daily by measuring optical density using a microplate reader Adsorbance 96 spectrophotometer (Byonoy, Germany) at λ = 605 nm and values were recorded by the software Absorbance 96. Wells containing YM 10% medium without fungal inoculation were used as blank for the OD readings. To calculate the change in OD 605, the blanks were subtracted from the OD 605 values of all tested strains. After incubation, 25 µL of each fungal culture were cultivated in duplicate in the presence of different HMs, individually, amending the isolation medium, using the following concentrations: 1000 ppm, 2500 ppm and 5000 ppm for Fe and Cu, and 100 ppm, 250 ppm and 500 ppm for Hg. Plates were then incubated at 4 °C for 15 days. Growth was evaluated daily as described below. Well containing YM 10% medium plus HM without fungal inoculation were used as blank for the OD readings.

4.2.1.5 Identification of fungal isolates

The fungal pure strains that were positive to the heavy metal's tolerance test were subjected to DNA extraction according to protocols previously described by Al-Hatmi et al. (2014). Briefly, from 1 to 10 mm^3 fungal material was transferred to a 2 mL Eppendorf tube containing 490 µL of CTAB-buffer 2x (2% CTAB, 1.4 M NaCl, 100 mM Tris pH 8) and acid washed glass beads. For mechanical breaking of hyphomycetes cells, the samples were homogenated manually with a sterile pestle for 5 minutes. Then 10 µL of Proteinase K (20 mg/mL) (Quiagen) was added and mixed thoroughly for 10 minutes. After incubation at 65 °C for 60 minutes, 500 µL of chloroform: isoamyl alcohol 24:1 (Sigma) were added and shacked for 2 minutes to form an emulsion. Subsequently, the samples were centrifuged for 10 minutes at 14.000 rpm and the aqueous upper layer was transferred to a new Eppendorf tube. After the addition of 2/3 of the volume of ice-cold pure isopropanol, the samples were centrifuged for 10 minutes at 14.000 rpm, and alcohol was removed. The pellet was then washed with 1 mL of ice cold 70 % ethanol (VWR Chemicals), air- dried and resuspended in 50 µL of Milli- Q water. The DNA was stored frozen at -20 °C for subsequently analyses. 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 Sybr Safe (1µl/25ml final concentration) (Invitrogen). The genomic DNA was PCR-amplifed with the universal primers ITS1/ITS4 which are specific for fungal interspacers ITS1, ITS2, and 5.8S rRNA genes (White et al, 1990). The reaction mixtures were prepared in a total reaction volume of 25 µL, containing 2 µL of template DNA, 5 µL of Buffer (Meridian Bioscientific), 1 µL of each primer (10µM) (Invitrogen), 0.5 µL of Taq polymerase (5U/µL) (Meridian Bioscientific). PCR was performed with the following thermocycling program: initial denaturation 5 minutes at 95 °C, followed by 35 cycles of 45 seconds at 95 °C, 30 seconds at 48 °C, 1 minute at 72 °C, and final extension was run at 72 C for 8 minutes (Marchetta et al., 2018) in a Mastercycler gradient (Eppendorf).

The results of the amplification reactions were analyzed by agarose gel electrophoresis (1.4 %, w/v) in TAE buffer (0.04 M Tris-acetate, 0.02 M acetic acid, 0.001 M EDTA), containing Syber Safe (1µl/25ml final concentration). Amplified products were purified using the QIAquick PCR purification kit (Qiagen), following the manufacturer's instructions. Sequencing was carried out at the EurofinsEurope (Germany). Sequences were then read and correct manually using FinchTV version 1.4 and reconstructed by MEGA11. Next relatives of isolates were determined by comparison to ITS 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).

4.2.1.6 Phylogenetic analysis

Sequences were further aligned using the program Clustal W to the most similar orthologous sequences retrieved from database. Each alignment was checked manually, corrected and then analyzed using the Neighbour-Joining method (Saitou & Nei, 1987) according to the model of Jukes-Cantor distances. A phylogenetic tree was constructed using the MEGA 11 (Molecular Evolutionary Genetics Analysis) software (Tamura et al., 2021). using the Maximum Composite Likelihood method (Tamura et al., 2004). The robustness of the inferred trees was evaluated by 1000 bootstrap re-samplings.

4.2.2 Metagenomic analysis

4.2.2.1 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. Through the use of 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, 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). Fungal internal transcribed spacer region 2 (ITS2) were amplified using the following primers: IlluAdp_ITS31_NeXTf 5′-CATCGATGAAGAACGCAG-3′ and IlluAdp_ITS4_NeXTr5′-TCCTSCGCTTAT TGATATGC-3′ (Guglielmin et al., 2023). Sequencing was performed using the Illumina MiSeq platforms, following the standard protocols of the company EurofinsEurope Services (Germany).

4.2.2.2 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.

Fungal taxonomy annotation was performed using ITS fungal database UNITE - Unified system for the DNA based fungal species linked to the classification (Nilsson et al., 2019) formatted for DADA2, offering an updated framework for annotating fungal taxonomy (unified system for the DNA based fungal species linked to the classification). Finally, a manual inspection was done, and sequences with abundance below of 0.1% were considered together in the minor groups of retrieved fungi.

4.2.3 Predictive Functional Profiling

Fungal function profiles were analysed using tool FUNGuild (Nguyen et al., 2016). FUNGuild v1.1 is a flat database hosted by GitHub (https://github.com/UMNFuN/FUNGuild), accessible for use and annotation by any interested party under GNU General Public License. The database currently contains a total of 9476 entries, with 66% at the genus level and 34% at the species level. The database is organised by entries into three broad groupings refered to as trophic modes (Tedersoo et al., 2014): (1) pathotroph = receiving nutrients by harming host cells (including phagotrophs); (2) symbiotroph = receiving nutrients by exchanging resources with host cells; and (3) saprotroph = receiving nutrients by breaking down dead host cells. Analyses were performed comparing the total ASV table results with the database, using a pyton3 command line as follows:

>Python3 FUNGuild.py taxa -otu example/otu_table.txt -format tsv -column taxonomy -classifier unite

>Python3 FUNGuild.py guild -taxa example/otu_table.taxa.txt

4.3 STATISTICAL ANALYSES

To compare the fungal 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 analysis (PCA) was performed using the factoextra R package, on data from selected physical and chemical properties of sediments and waters, and the relative abundance of significant fungal groups. 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 connection among retrieved factors and environmental parameters, results were considered significant when the *P* value was less than 0.05. Prior to this analysis, the data were log-transformed to linearize the relationships and avoid the influence of magnitude.

4 RESULTS

5.1 CULTURAL ANALYSES

5.1.1 Abundance and morphotype of fungi isolated from water and sediment samples

Water

Results of fungal abundance in water samples of Arctic lakes are reported in Table 3. The highest abundance of fungi was obtained for samples taken from Lake Solvannet (L1), with counts ranging from 81 CFU/100 mL in L1-2, to 51.5 CFU/100 mL L1-1. The lowest abundance was observed for Lake Knudsenheia (L3), where values from 2.5 CFU/100 mL up to 3.75 CFU/100 mL were detected. In general, a comparable abundance of fungi was obtained in the two media used for almost all sampling sites; exception was the sampling site of Lake Glacier (L2) that showed higher counts on YM medium respect to DRBC medium, especially for site L2-1 (38 CFU/100 mL *vs.* 17 CFU/100 mL) and site L2-2 (44.5 CFU/100 mL *vs.* 16.25 CFU/100 mL). In the Storvatnet (L4) and Tvillingvatnet (L5) lakes, the number of fungal colonies were ranging from 6.5 (L5-1) to 15 (L4-3).

A total of 229 fungal strains (105 yeasts and 124 hyphomycetes) were isolated from water samples collected from Arctic lakes. Among yeasts, the most frequently isolated morphotype was represented by white yeast colonies which turn to pink (55.24%), followed by red yeast strains (27.61%) and beige-whitish yeasts (17.14%). Out of 124 filamentous strains, the most representative isolates were dark hyphomycetes (18.55 %), white hyphomycetes (12. 90%) and pinkish hyphomycetes (9.68%) Identification of these isolates is still in progress.

Table 3. Viable counts (mean ±standard deviation) of water samples collected from Arctic lakes.

Lake sampling site	DRBC	YM
	Viable counts (CFU/100 mL)	Viable counts (CFU/100 mL)
L1-1	5.15E+01 ± 4.95E+00	6.15E+01 ± 2.33E+01
L1-2	8.10E+01 ± 2.69E+01	7.70E+01 ± 9.19E+00
L1-3	5.25E+01 ± 7.78E+00	6.40E+01 ± 5.66E+00
L2-1	1.70E+01 ± 1.41E+00	3.80E+01 ± 1.41E+00
L2-2	1.63E+01 ± 2.47E+01	4.45E+01 ± 3.89E+01
L2-3	1.50E+01 ± 1.41E+00	2.20E+01 ± 1.41E+00
L3-1	3.75E+00 ± 7.07E-01	2.50E+00 ± 1.41E+00
L3-2	2.75E+00 ± 2.12E+00	2.50E+00 ± 0.00E+00
L3-3	3.00E+00 ± 0.00E+00	3.50E+00 ± 7.07E-01
L4-1	1.05E+01 ± 3.54E+00	1.00E+01 ± 1.41E+00
L4-2	1.30E+01 ± 0.00E+00	1.05E+01 ± 2.12E+00
L4-3	1.03E+01 ± 4.95E+00	1.50E+01 ± 2.83E+00
L5-1	n.d. n.d.	6.50E+00 ± 7.07E-01
L5-2	n.d. n.d.	1.20E+01 ± 4.24E+00
L5-3	n.d. n.d.	1.15E+01 ± 7.07E-01

n.d.: for lake L5 the cultural analysis of water samples was carried out only on YM agar medium.

Results of fungal abundance in water samples of Antarctic lakes are reported in Table 4. In general, a lower abundance was obtained from Antarctic lake samples respect to Arctic samples, with counts ranging from 42 CFU/100 mL (LB-3) to 0.5 CFU/100 mL (LS-2 and LS-3). No CFU were observed for Lake Extremadura (LE). The highest abundance was obtained for Lake Ballaneros (LB) on YM agar medium with 40.5 CFU/100 mL, 24 CFU/100 mL and 42 CFU/100 mL in samples LB-1, LB-2 and LB-3 respectively. For Lake Argentina (LA) counts ranged from 13 CFU/100 mL in LA-3 to 0.75 CFU/100 mL in lake in LA-1. Viable counts of water samples of Lake Sofia were higher on YM medium, with values ranging from 3.5 CFU/100mL (LS-3) to 11.5 CFU/100mL (LS-2) respect to DRBC medium where the fungal colonies ranged from 2.0 CFU/100mL (LS-1) to 0.5 CFU/100mL (LS-2 and LS-3). Comparable results were obtained for Crater (LC), Telefon (LT) and Zapatilla (LZ) lakes, where abundance was between 1.5 CFU/100 mL to 6.5 CFU/100 mL.

A total of 69 strains (18 yeasts and 51 hyphomycetes) were isolated from water samples coming from Antarctic lakes. Yeasts were represented by pink (11), white (6) and red (1) isolates. The most frequent morphotypes isolated for filamentous fungi were white hyphomycetes, white hyphomycetes with yellow reverse and dark green hyphomycetes, each group representing the 21.57% of filamentous isolates. Identification of these strains is still in progress.

Table 4. Viable counts (mean ±standard deviation) of water samples collected from Antarctic lakes.

Lake sampling site	DRBC	YM
	Viable counts (CFU/100 mL)	Viable counts (CFU/100 mL)
LA-1	0.00E+00 ± 0.00E+00	7.50E-01 ± 2.12E+00
LA-2	1.00E+00 ± 1.41E+00	4.50E+00 ± 6.36E+00
LA-3	9.00E+00 ± 1.13E+01	1.30E+01 ± 1.41E+00
LB-1	1.33E+01 ± 7.07E-01	4.05E+01 ± 3.54E+00
LB-2	3.25E+00 ± 3.54E+00	2.40E+01 ± 1.41E+00
LB-3	1.00E+01 ± 1.41E+00	4.20E+01 ± 0.00E+00
LC-1	1.50E+00 ± 7.07E-01	2.00E+00 ± 2.83E+00
LE-1	0.00E+00 ± 0.00E+00	0.00E+00 ± 0.00E+00
LS-1	2.00E+00 ± 1.41E+00	6.00E+00 ± 2.83E+00
LS-2	5.00E-01 ± 7.07E-01	1.15E+01 ± 6.36E+00
LS-3	5.00E-01 ± 7.07E-01	3.50E+00 ± 2.12E+00
LT-1	3.25E+00 ± 4.95E+00	3.75E+00 ± 2.12E+00
LT-2	4.00E+00 ± 1.41E+00	6.50E+00 ± 7.07E-01
LT-3	4.00E+00 ± 1.41E+00	4.50E+00 ± 7.07E-01
LZ-1	2.50E+00 ± 2.12E+00	2.50E+00 ± 2.12E+00
LZ-2	3.00E+00 ± 1.41E+00	4.50E+00 ± 2.83E+00

Sediment

No enumeration was carried for any of the samples examined due to the low number of fungal colonies found in sediments both from Artic and Antartic lakes. In fact, only 5 colonies were isolated from 3 sites (two colonies from L1-1, one colony from L2-3 and two colonies from L5-3) of 3 different Arctic lakes. Three strains were white filamentous fungi, while two strain were yeasts.

Also, in the sediments from Antarctica the presence of fungal isolates was occasional. A total of 24 colonies were isolated from 9 sites (four colonies from LA-1, three colonies from LB-1, one colony from LB-2, two colonies from LC-1, four colonies from LS-1, two LS-3, seven LT-3, LZ-2, LE-1) of 7 different Antarctic lakes. The majority of the strains were white filamentous fungi.

5.1.2 HM tolerant fungal strains isolated from enrichment cultures

Results obtained from the isolation of fungal strains from sediment culture enriched with HMs Fe, Cu and Hg are summarized in Table 5. A total of 51 fungal strains (31 filamentous fungi and 20 yeasts) were isolated. The majority of strains were isolated from Fe enrichments (32 isolates), followed by Hg enrichments (16 isolates). Only 3 strains were obtained for Cu enrichments, isolated from Lake Glacier (L2). Interesting to note that the most of fungal colonies were obtained from Lake Ballaneros (LB) in Antarctica (14 isolates).

Table 5. Number of fungal isolates *per* polar lake for each enrichment

Lake	Enrichment		
	Fe 1000 ppm	Cu 1000 ppm	Hg 100 ppm
L1	5	0	0
L2	1	3	0
L3	2	0	3
L4	1	0	0
LA	3	0	1
LB	6	0	8
LC	4	0	0
LE	0	0	0
LS	3	0	0
LT	3	0	4
LZ	4	0	0

5.1.3 Positive fungal strains for tolerance to increasing HM concentrations

The fifty-one fungi isolated from natural enrichment were further screened for tolerance to higher concentrations of the same HM used in the isolation medium at 4 °C. The HMs concentrations used were: 2500 ppm and 5000 ppm for Fe and Cu; 250 ppm and 500 ppm for Hg. A total of 18 isolates (12, 5 and 1 for Fe, Hg and Cu respectively) (Table 6) were considered positive due to their ability to grow at the higher HM concentration tested (Figure 3). Among them, 6 isolates were from the Arctic region and 12 from Antarctic area (Table 6).

Table 6. Heavy metal tolerant fungal strains and their provenience.

Strain ID	Sample origin		
L2-3CCua	Lake Glacier	Ny-Ålesund	Arctic
L1-2CFeb	Lake Solvannet		
L1-1CFea			
L3-1CFea	Lake Knudsenheia		
L3-2CFea			
L3-2CHga			
Y-AAA2	Lake Argentina	Livingston Island	Antarctica
Y-AAA4			
AAC1			
Y-ASA2	Lake Sofia		
Y-ASA3			
Y-ASA4			
ABC3	Lake Ballaneros	Deception Island	
Y-ABC1			
Y-ABC2			
Y-ABA1			
Y-AZA1	Lake Zapatilla		
Y-AZA2			

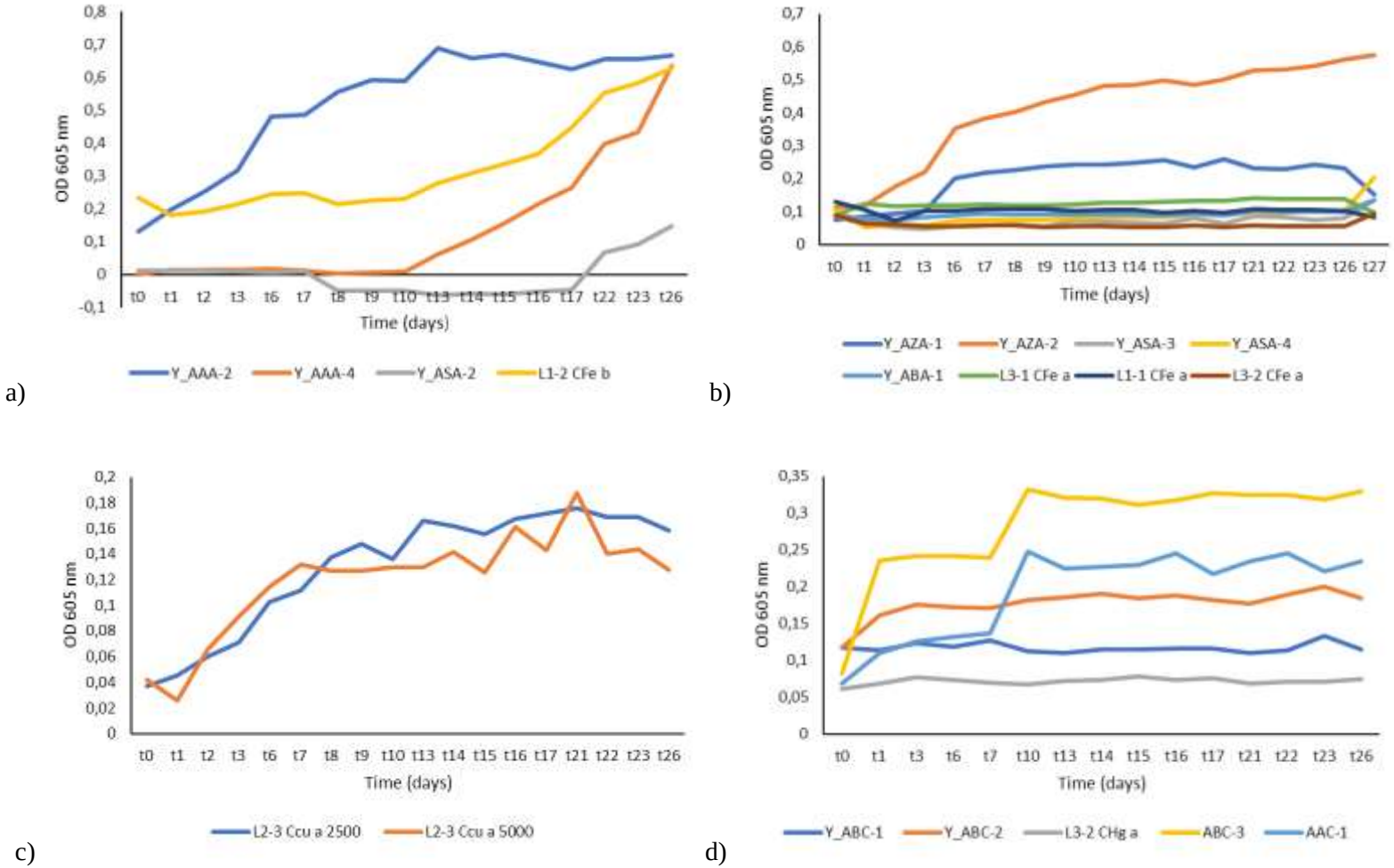


Figure 3. Fungal growth with the highest concentrations of HMs. Optical density (605 nm) is plotted against time (days). a) Growth of filamentous fungal isolates at Fe 5000 ppm; b) growth of yeast isolates at Fe 5000 ppm; c) growth of one fungal isolate at Cu 2500 ppm and Cu 5000 ppm; d) growth of 5 yeast isolates at Hg 500 ppm

Among Artic isolates, three positive high HM tolerant fungal strains (L3-1CFea, L3-2CFea tolerant to high concentration of Fe and L3-2CHga tolerant to high concentration of Hg) were isolated from Lake Knudsenheira; two positive high Fe tolerant strains (L1-2CFeb and L1-1CFea) were isolated from Lake Solvannet and the only one strain (L2-3CCua) that resulted positive at higher concentration of Cu was isolated from Lake Glacier.

Among Antarctic isolates, four HM tolerant fungi (ABC3, Y-ABC1, Y-ABC2 tolerant to high concentration of Hg and Y-ABA1 tolerant to high concentration of Fe) were isolated from the Lake Ballaneros in Deception Island; three strains tolerant to high concentration of Fe (Y-ASA2, Y-ASA3 and Y-ASA4) were isolated from Lake Sofia and three strains (YAAA2, Y-AAA4 tolerant to high concentration of Fe and AAC1 strain tolerant to high concentration of Hg) from Lake Argentina (Livingston Island). Two strains (Y-AZA1 and Y-AZA2) both tolerant to high concentration of Fe were isolated from Lake Zapatilla.

5.1.4 Multi-tolerant fungal strains

Fungal strains which tolerated high concentration of HMs were further tested for growth at 4 °C in the presence of metals different from that amending the isolation medium. Results are shown in Table 7.

None of the 18 strains tested was able to tolerate the high concentrations of all metals (Fe, Cu and Hg) tested. However, one fungal strain (Y-AAA4) tolerant to high concentration of Fe (5000ppm) was able to grow in presence of high concentration of Hg (500 ppm). Another fungal strain (L2-3 CCua) tolerant to high concentration of Cu (5000ppm) and 5 isolates (L3-2CHga, AAC-1, Y-ABC-1, Y-ABC2, ABC3) tolerant to high concentration of Hg (500ppm) showed growth with high concentration of Fe (5000ppm).

Table7. List of fungal isolates and respective HMs tolerated.

Strain	Lake	Heavy metals (ppm)								
		Fe			Cu			Hg		
		1000	2500	5000	1000	2500	5000	100	250	500
L1-1 CFea	L1	+	+	+						
L1-2 CFeb	L1	+	+	+						
L3-1 CFea	L3	+	+	+						
L3-2 CFea	L3	+	+	+						
Y-AAA2	LA	+	+	+						
Y-AAA4	LA	+	+	+				+	+	+
Y-ABA1	LB	+	+	+						
Y-ASA2	LS	+	+	+						
Y-ASA3	LS	+	+	+						
Y-ASA4	LS	+	+	+						
Y-AZA1	LZ	+	+	+						
Y_AZA2	LZ	+	+	+						
L2-3 CCua	L2	+	+	+	+	+	+			
L3-2 CHga	L3	+	+	+				+	+	+
AAC-1	LA	+	+	+				+	+	+
Y-ABC1	LB	+	+	+				+	+	+
Y-ABC2	LB	+	+	+				+	+	+
ABC3	LB	+	+	+				+	+	+

5.1.5 Molecular dentification and phylogenetic analysis

All 18 HM positive strains were identified using the ITS DNA target, furthermore two additional strains Y-ACA2 andY-ATA3 tolerant to 1000 ppm of Fe were also identified due to their interesting and particular phenotype (Figure 4). In particular, Y-ACA2, a filamentous isolate with white mycelium, changed the color of YM medium from pale yellow to violet (Figure 4a). Y-ATA3 was a yeast-like black fungus with slow growth (Figure 4b).

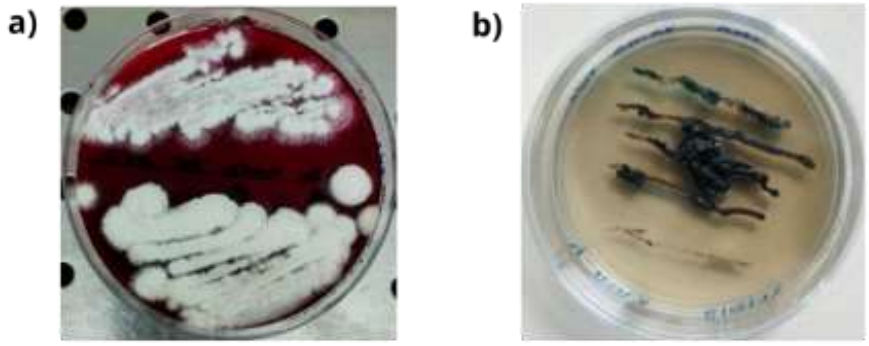


Figure 4. (a) Strain Y-ACA2 growth on YM agar after 15 days of incubation at 4 °C; (b) Y-ATA3 growth on YM agar after 20 days of incubation at 15 °C.

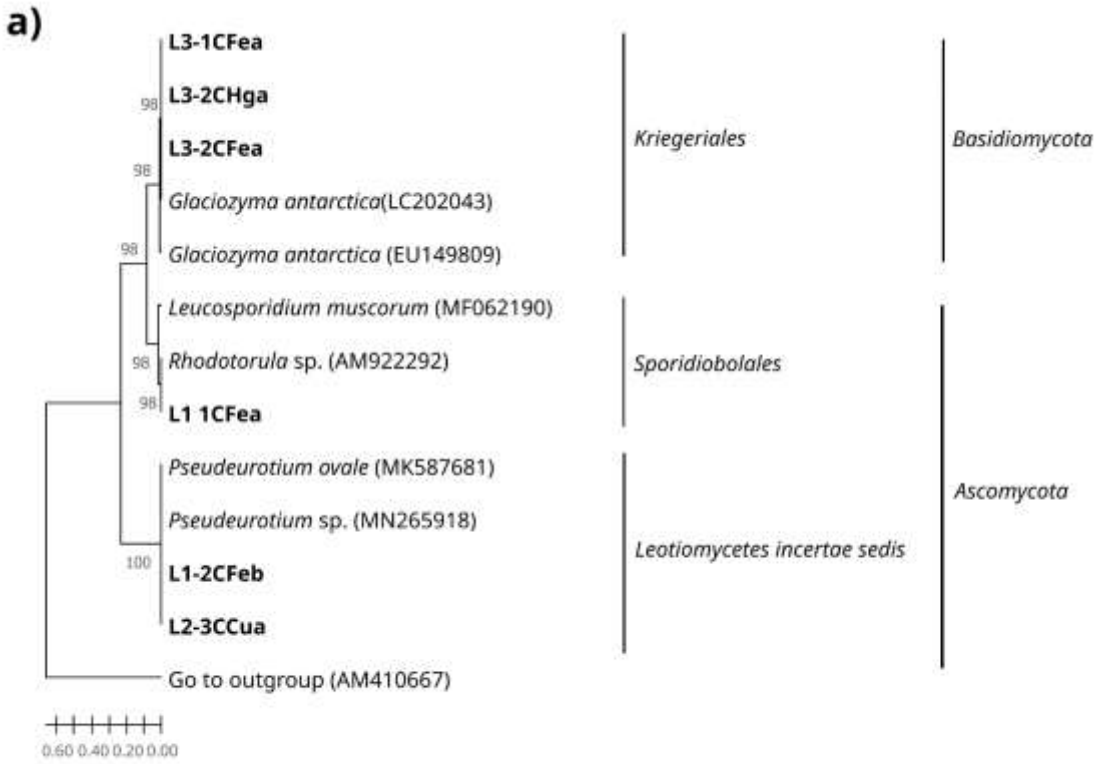
All 20 isolates were successfully amplified and phylogenetically identified (Table 8). HM tolerant fungal isolates mainly belonged to the phylum of Basidiomycota (4 and 9 isolates from Arctic and Antarctic samples, respectively), followed by the phylum Ascomycota (2 and 5 isolates from Arctic and Antarctic samples, respectively). The majority of the strains. belonging to Basidiomycota. were mostly related to the order of Kriegeriales with the family Camptobasidiaceae. Other HM tolerant basidiomycetous fungi coming from Artic lakes belonged to the order of Sporidiobolales with the family of Sporidiobolaceae. Instead, HM tolerant basidiomycetous fungi of the order of Holtermanniales, Cystofilobasidiales and Tremellales and the family Mrakiaceae and Cryptococcaceae have been isolated from Antarctic lakes. Concerning the HM tolerant Ascomycota coming from Artic lakes they belonged exclusively to Leotiomycetes *incertae sedis* order with the family of Pseudeurotiaceae.

The Antarctic HM tolerant fungi were instead affiliated to three different Ascomycota orders: Helotiales, Leotiomycetes *incertae sedis* and Chaetothyriales and three respectivelly different family: Helotiales incertae sedis, Pseudeurotiaceae and Herpotrichiellaceae. The identification at species level was obtained for 12 strains. Two strains were identified at order level, and 6 at genus level (Table 8).

Table 8. Taxonomic classification of HM tolerant fungi based on rDNA ITS genes, separated for Arctic and Antarctic sites.

Sample origin	Sample ID	Nearest organism									Ref.
		Affiliated organisms	Affiliation %	Accession Number	Phylum	Subphylum	Class	Order	Family	Genus	
Arctic	L1-2CFeb	*Pseudeurotium* sp.	100	MN265918	*Ascomycota*	*Pezizomycotina*	*Leotiomycetes*	*Leotiomycetes incertae sedis*	*Pseudeurotiaceae*	*Pseudeurotium*	Santos et al., 2020
	L2-3CCua	*Pseudeurotium* sp.	99.81	MN265918							
	L1-1CFea	*Rhodotorula* sp.	98.96	AM922292				*Sporidiobolales*	*Sporidiobolaceae*	*Rhodotorula*	Pathan et al., 2010
	L3-1CFea	*Glaciozyma antarctica*	98.1	EU149809	*Basidiomycota*	*Pucciniomycotina*	*Microbotryomycetes*	*Kriegeriales*	*Camptobasidiaceae*	*Glaciozyma*	Connell et al., 2008
	L3-2CFea	*Glaciozyma antarctica*	98.06	EU149809							
	L3-2CHga	*Glaciozyma antarctica*	98.22	EU149809							
Antarctica	Y-AAA2	*Cadophora fastigiata*	100	MT635284	*Ascomycota*	*Pezizomycotina*	*Leotiomycetes*	*Helotiales*	*Helotiales incertae sedis*	*Cadophora*	Cudowski and Świsłocka, 2022
	Y-AAA4	*Helotiales* sp.	99.62	MF043974							Singh et al., 2020
	Y-ASA2	*Helotiales* sp.	99.62	MF043974							
	Y-ACA2	*Pseudogymnoascus* sp.	100	MH790448				*Leotiomycetes incertae sedis*	*Pseudeurotiaceae*	*Pseudogymnoascus*	Albores et al., 2018
	Y-ATA3	*Exophiala* sp.	100	ON935448			*Eurotiomycetes*	*Chaetothyriales*	*Herpotrichiellaceae*	*Exophiala*	
	ABC3	*Holtermanniella wattica*	100	JQ857031	*Basidiomycota*	*Agaricomycotina*	*Tremellomycetes*	*Holtermanniales*	*Holtermanniales incertae sedis*	*Holtermanniella*	Carrasco et al., 2012
	Y-ABC1	*Holtermanniella wattica*	100	JQ857031							
	Y-ABC2	*Holtermanniella wattica*	100	JQ857031							
	Y-AZA1	*Mrakia robertii*	100	MT048630				*Cystofilobasidiales*	*Mrakiaceae*	*Mrakia*	Sanyal et al., 2020
	Y-AZA2	*Cryptococcus* sp.	100	KU145523				*Tremellales*	*Cryptococcaceae*	*Cryptococcus*	Troncoso et al., 2017
	AAC1	*Glaciozyma watsonii*	100	NR_132821		*Pucciniomycotina*	*Microbotryomycetes*	*Kriegeriales*	*Camptobasidiaceae*	*Glaciozyma*	Connell et al., 2008
	Y-ABA1	*Glaciozyma martinii*	100	MT048654							Sanyal et al., 2020
	Y-ASA3	*Glaciozyma watsonii*	100	MT048654							
	Y-ASA4	*Glaciozyma watsonii*	100	MT048654							

Based on these results and using the reference sequences (downloaded from NCBI database) related to our sequences, two phylogenetic trees were constructed (Figure 5 a and b).



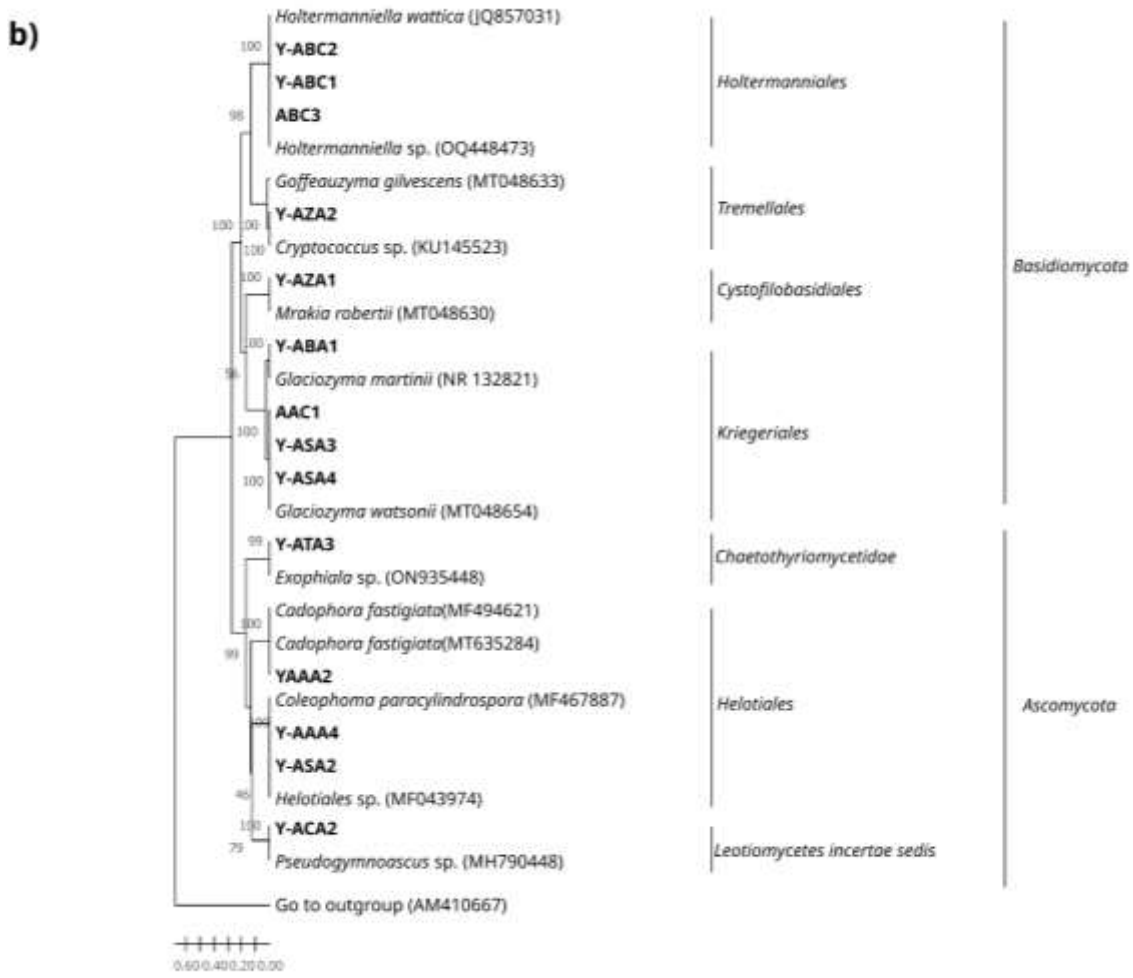


Figure 5. Phylogenetic trees based on ITS region of selected strains. The evolutionary history was inferred using the UPGMA method (Sneath &Sokal, 1973). The bootstrap consensus tree inferred from 1000 replicates (Felsenstein, 1985) is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test are shown next to the branches. The evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura et al., 2004) and are in the units of the number of base substitutions per site. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Evolutionary analyses were conducted in MEGA11 (Tamura et al., 2021). a) tree constructed with Arctic positive strains sequences; b) tree constructed with Arctic positive strains sequences. Our strains sequences are reported in the tree in bold.

The tree of Arctic sequences showed two main branches (Figure 5a), one with that is subsequently divided in other two parts representing the orders Kriegeriales and Sporidiobolales. In the first cluster of the branch there are the genus *Glaciozyma* with tree of strains affiliated (L3-1CFea, L3-2CHga and L3-2CFea). The second cluster (in order of

Sporidiobolaes) is mainly rapresented by genera *Leucosporidium* and *Rhodotorula* with the strain L1-1CFea. Finally, the second branch represent the order Leotiomycetes incertae sedis showed the genus *Pseuderotium* with how are related our strains L1-2CFeb and L2-3CCua.

The Tree constructed with Antarctic sequences (Figure 5 b) is divided in two principal branches that representing exactly the two retrieved phyla (Basidiomycota and Ascomycota). The first branch is subsequently subdivided in other two groups, one with the orders Holtermanniales and Tremellales, and the second with the orders Cystofilobasidiales and Kriegeriales. The first group is represented by genus of *Holtermanniella* with which three of our isolates (Y-ABC2, Y-ABC1 and ABC3) showed affiliation, and the genera *Goffeauzyma* and *Cryptococcus* which showed a strict relation with the isolate Y-AZA2. In the second group were present the genera *Marakia* related to our strain YAZA1, and *Glaciozyma* with which four of our strains showed a strict relation (Y-ABA1, AAC1, Y-ASA3 and Y-ASA4). The second branch (Ascomycota phylum) was also divided in two principal clusters, with the first represented by the family of Chaetothyriomycetidae and the genus *Exophiala* related to one of our fungi (strain Y-ATA3). In the second cluster were present the orders Helotiales and Leotiomycetes incertae sedis, mainly represented by genera *Coleophoma, Helotiales*, and *Pseudogymnoascus* affiliated to the isolates Y-AAA4, Y-ASA2 and Y-ACA2.

Particularly the two strains added Y-ATA3 (melanized strain) and Y-ACA2 (red-violet pigment producer) were affiliated to the genera *Exophyala* sp. and *Pseudogymnoascus* sp., respectively.

5.2 CULTURE-INDEPENDENT RESULTS

5.2.1 Metagenomic results of environmental samples

For metagenomics were analyzed 20 environmental samples (Table 9). Samples from LE and LC were excluded cause of no HM tolerant strains were isolated from them. Furthermore, two of the sequenced samples had not good results in the first enrichment steps which caused a creation of a low-quality library and subsequently the impossibility to continue with the NGS sequencing. This was probably due to low ITS DNA quantity, also if the total extracted DNA showed high concentration and good quality (Table 9).

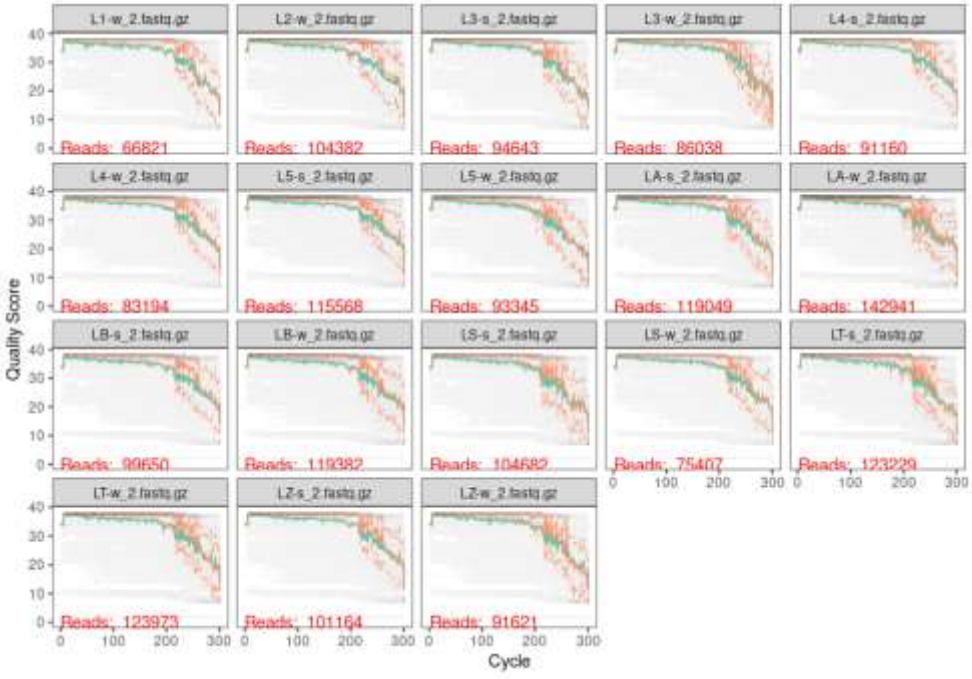
Table 9. Concentration and quality of DNA extracted from water and sediment samples.

Sample Name	Nucleic Acid Conc(ng/μL)	OD260/280	OD260/230
L1-s	92.7	2.05	1.2
L2-s	18.31	2.22	1.4
L3-s	16	1.95	1.7
L4-s	68.76	1.81	1.3
L5-s	50.97	1.75	2.04
LA-s	12.24	1.91	1.07
LB-s	25.39	1.71	1.7
LS-s	28.13	2.25	1.25
LT-s	15.81	1.84	2.41
LZ-s	14	1.98	1.21
L1-w	22.59	1.99	1.25
L2-w	27.73	2.05	2.06
L3-w	24.34	2.16	1.9
L4-w	42.65	2.04	1.52
L5-w	20	2	2.33
LA-w	24.45	2.13	1.25
LB-w	7.19	2.73	2.04
LS-w	13.25	2.54	2.06
LT-w	12.64	2.16	2.03
LZ-w	37.27	1.85	1.66

In particular missing samples were sediment in L1 and L2 (Svalbard Islands). Overall, a preliminary analysis of raw sequences showed a total of 1836249 paired reads. The quality of

reads is reported in Figure 6, where is possible to appreciate a quality of reads R1 greater than 30 of quality score in all samples and for the entire read length (Figure 6 a). Differently, quality of R2 reads decreased at around 250 nt length when it drops below 20 quality score (Figure 6 b).

a)



b)

Figure 6. Sequences quality before cleaning steps, on the x axis is reported the reads length, and on the y axis the quality score value of the bases. a) showed the R1 reads; b) showed the R2 reads.

Overall, also the error rate in the raw reads (Figure 7) show a typically situation driven by a very high density of maximum quality score bases, which interacts with the weighted loess-score error-model fitting.

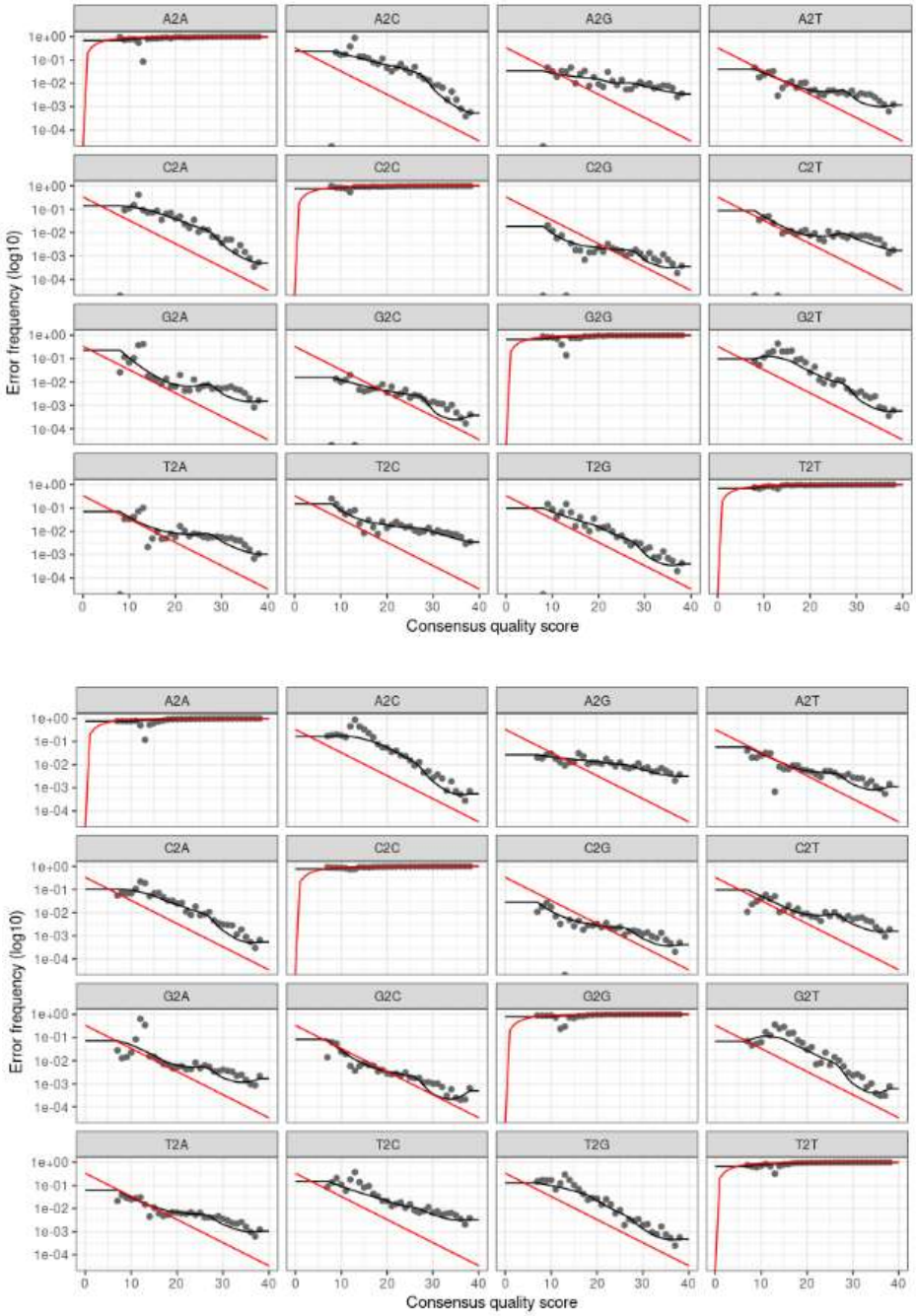


Figure 7. The figures report the error rate assessed by the DADA2 R commands "learnErrors"and "plotErrors". In the right image are reported the error rates for R1 reads, and in the second the error rates of R2 reads.

Based on the preliminary analyses, reads have been cleaned and merged using DADA2 R package (Table 10). Finally, for all samples were recovered more than 85% of the initial sequences as good quality sequences, except for sample L2-w which showed 82.77% of final good quality reads. The obtained good quality reads were than used for subsequent bioinformatics analyses.

Table 10. Preliminary data table of sequences obtained by Illumina MySeq 300 bp paired end.

sample	input"	filtered	denoiseR1	denoisedR2	merged	nonchim	% of Good quality sequences
L1-w	66821	60317	59983	60018	59868	59033	88.34
L2-w	104382	94337	93333	93499	93014	86401	82.77
L3-s	94643	86809	86051	86237	85796	84902	89.71
L3-w	86038	78617	78315	78340	78172	77100	89.61
L4-s	91160	83540	82682	82612	82174	81784	89.71
L4-w	83194	76345	75352	75427	74940	74201	89.19
L5-s	115568	106339	105173	105179	104640	103146	89.25
L5-w	93345	84443	83651	83658	83346	80877	86.64
LA-s	119049	108673	107907	107998	107535	105979	89.02
LA-w	142941	131313	130885	131193	130810	130225	91.10
LB-s	99650	90725	90080	90105	89682	89202	89.52
LB-w	119382	109010	108585	108562	108321	107303	89.88
LS-s	104682	95884	95339	95313	94986	91525	87.43
LS-w	75407	69190	68965	69026	68827	67871	90.01
LT-s	123229	112667	112500	112539	112406	112109	90.98
LT-w	123973	113979	113682	113785	113570	112848	91.03
LZ-s	101164	93354	92920	92980	92646	92236	91.17
LZ-w	91621	83002	82670	82681	82514	82332	89.86

The analyses of sequences continued by DADA2 Amplicon Sequences Variant approach (ASV). Starting from the ASVs table generated after bioinformatics analyses, the fungal community composition was evaluated for lakes' sediment and water samples.

5.2.2 Alpha diversity influence of environmental parameters

The diversity indices were calculated for each water and sediment sample, based on final ASVs tables obtained after bioinformatics analyses. (Table 11).

Table 11. Diversity indices calculated using the total retrieved ASVs.

Polar region	matrice	sample	Observed	Chao1	ACE	Shannon	Simpson	InvSimpson	Fisher
Arctic	Water	L1-w	274	274.38	275.00	2.89	0.88	8.38	37.17
		L2-w	563	563.40	564.21	4.80	0.98	53.50	80.69
		L3-w	184	185.75	186.92	1.72	0.57	2.34	22.62
		L4-w	585	587.02	589.17	4.10	0.95	19.78	86.61
		L5-w	470	471.49	473.39	2.99	0.86	7.40	66.10
	Sediment	L3-s	461	461.63	461.93	3.17	0.83	5.80	64.12
		L4-s	645	645.32	645.81	4.41	0.96	26.24	95.50
		L5-s	885	885.68	886.39	4.69	0.97	29.46	132.98
Antarctic	Water	LA-w	102	111.55	113.20	2.45	0.85	6.58	10.86
		LB-w	489	489.08	489.32	4.01	0.95	19.44	66.15
		LS-w	390	390.75	390.92	4.82	0.98	43.99	54.75
		LT-w	222	222.00	222.00	3.34	0.89	9.44	26.57
		LZ-w	243	244.91	244.85	2.55	0.83	5.83	30.79
	Sediment	LA-s	636	636.53	637.06	3.98	0.92	13.33	89.92
		LB-s	409	409.06	409.39	3.87	0.95	20.38	55.38
		LS-s	481	481.33	481.97	4.49	0.97	30.32	66.56
		LT-s	186	186.00	186.00	2.79	0.85	6.68	21.76
		LZ-s	398	398.13	398.37	3.80	0.94	15.41	53.39

Generally, it is possible to observe that lakes showed a great variability, and there was not a unique trend. Sediments showed slightly higher diversity values if compared with water (e.g. Shannon diversity average 3.4 and 3.9 for water and sediments, respectively). The highest value of Shannon index was found in water sample of LS (Livingston Island, Antarctica) and a comparable value was found also in the sediment of the same lake (4.49). Instead, the lowest value of Shannon diversity was retrieved in the water sample of L3 (Svalbard Island, High Arctic), and also sediments of the same lake showed a value lower than the average sediments' Shannon index value (3.17).

If in terms of diversity, analyzed lakes didn't show significant trends, the analyses of environmental parameters showed a completely different situation (Figure 8).

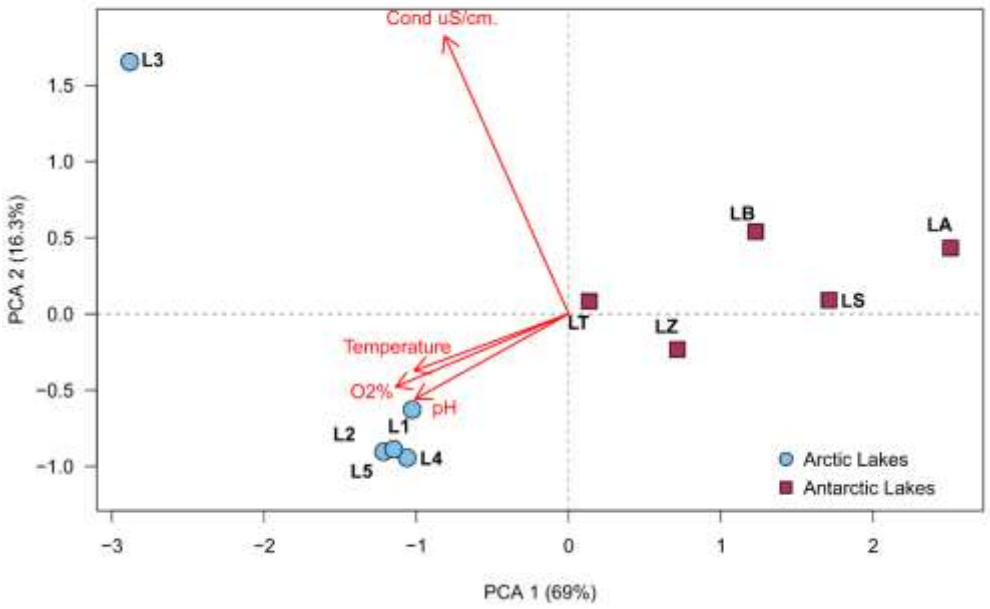


Figure 8. Principal component analysis obtained by recorded environmental parameters, made by factoextra R package.

In the principal component analysis, it was possible to observe that lakes were completely separated by the environment, in fact Arctic and Antarctic samples were completely distinct and generated two different groupings, with the only exception represented by Arctic lake L3. This lake was strictly related to the conductibility, probably due to its closeness to the sea cost and therefore it is considerably influenced by salt water.

5.2.3 ASVs distribution and differences

The obtained ASVs table was used to evaluate whether the differences highlighted by the principal component analysis of the environmental parameters also had repercussions within the retrieved amplicon sequences variants (ASVs).

In Figure 9, it is possible to appreciate that also phylogenetic groups showed an almost complete separation between Arctic and Antarctic regions. Overall, a total of 5980 ASVs have been retrieved, and of these only the 1.7% (102 ASVs) were shared between Arctic and Antarctic samples.

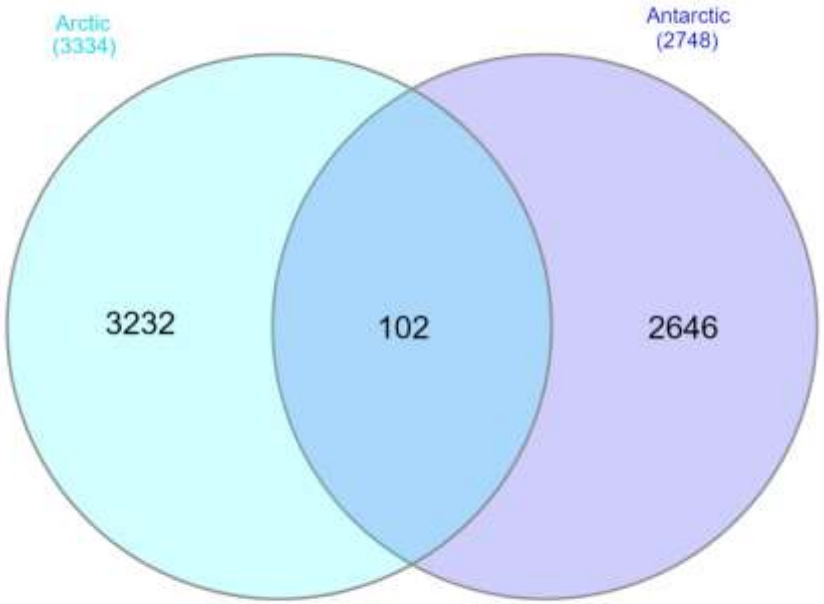


Figure 9. Venn diagram generated using all the retrieved ASVs separated by region of origin (Arctic and Antarctic). The diagram was made by InterctiVeen online tool (Heberle et al., 2015).

Similarly, ASVs table was used to evaluate differences between all water and all sediments samples, trying to understand if lakes showed similar population, or water and sediments have different inhabitants. In Figure 10 it is possible to appreciate that, in this case, the percentage of common ASVs between water and sediment samples were higher than that underlined between Arctic and Antarctic, showing a value of 10.87%. This is probably due to the fact that more ASVs were shared between water and sediment samples from the same region, or from the same lake.

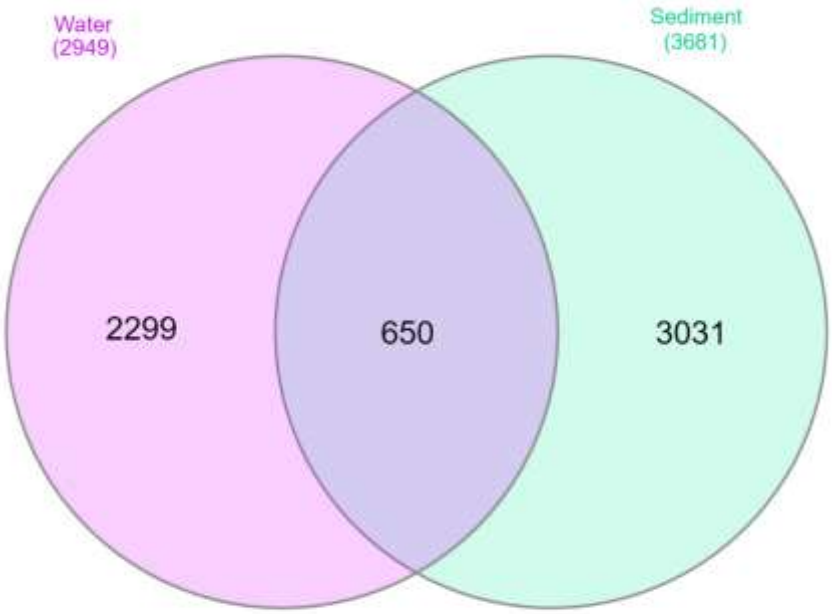


Figure 10. Venn diagram generated using all the retrieved ASV separated by matrix of origin (water and sediment). The diagram was made by InterctiVeen online tool (Heberle et al., 2015).

Finally, following the above results, the ASVs were examined separated by region (Arctic and Antarctic) and by matrix (water and sediments). Results are shown in Figure 11.

This investigation showed what it was supposed above, in fact, Arctic *vs* Antarctic water and Arctic *vs* Antarctic sediments showed really low percentage of common ASVs (1.79 and 1.16%, respectively), in agreement with what was seen for the total ASVs compared by region of origin. Differently, water *vs* sediment from Arctic and water *vs* sediment from Antarctic region, shared higher number of ASVs (8.36 and 13.28% respectively). Furthermore, more similarity was observed between sequences retrieved from Antarctic than those from Arctic region.

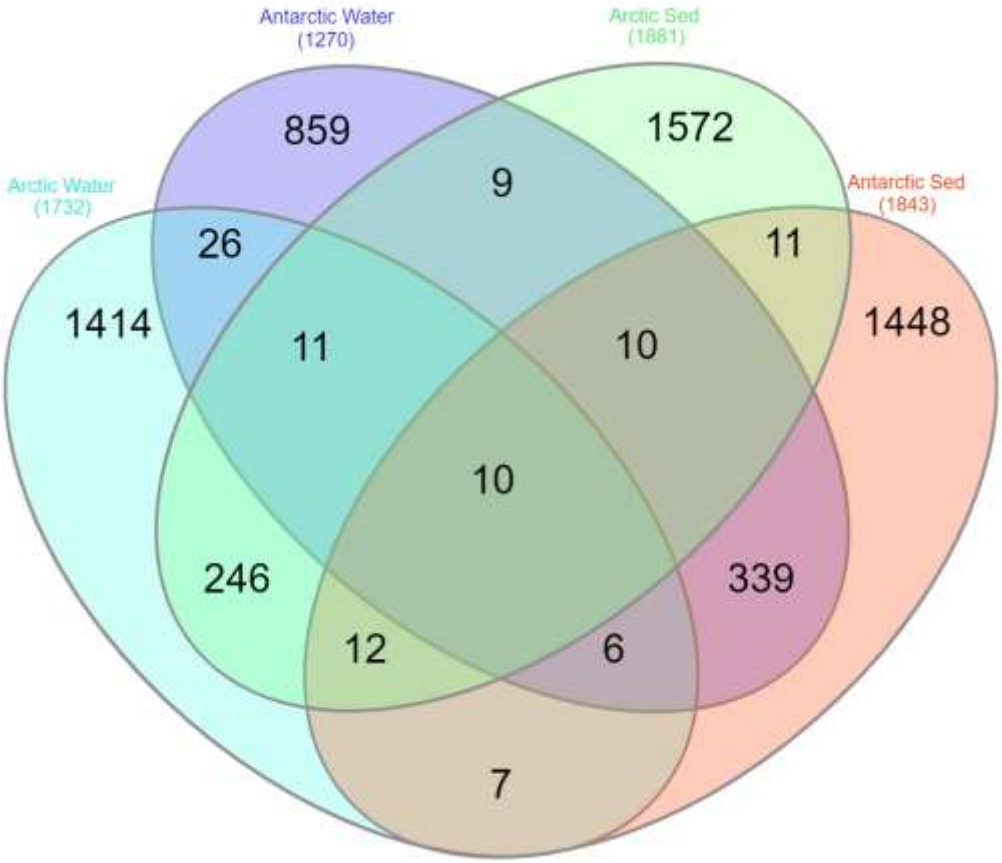


Figure 11. Venn diagram generated using all the retrieved ASVs separated by region (Arctic and Antarctic) and matrix of origin (water and sediment). The diagram was made by InterctiVeen online tool (Heberle et al., 2015).

5.2.4 Fungal phyla distribution in lakes' water and sediment

Starting from the ASVs table generated after sequencing, the fungal community composition in each sample was determined. In Arctic lakes (Figure 12) the analysis of phyla showed that almost in all samples an average of 50% ASVs were related to Fungi_phy_Incertae_sedis. The most represented and almost equally distributed phylum was represented by Chytridiomycota, with an average percentage of 24%. The highest value was observed in water samples of the L4 (46.2%) and the lowest value in L5 water (4.9%). Rozellomycota was the second most represented phylum, but with an uneven distribution: the highest abundance value was 20.5% (sediments of L4) and the lowest was 0.16% (water of L1).

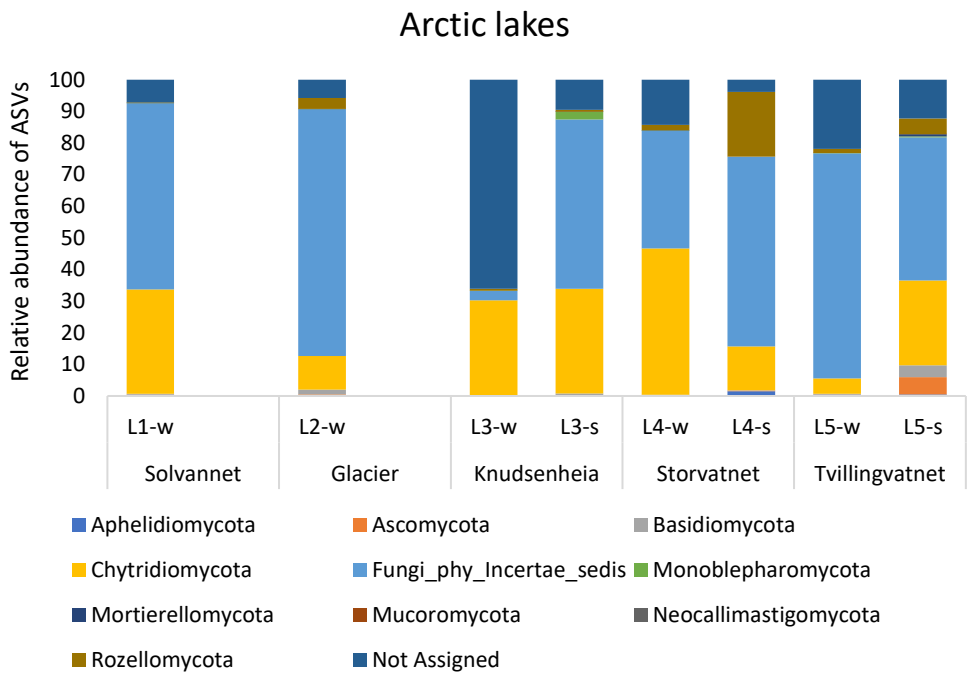


Figure 12. Fungal community structure at phylum level, in water and sediment samples from Arctic lakes.

The phyla Ascomycota and Monoblepharomycota were retrieved with percentage higher than 1% only in L5 sediments (5.49%) and L3 sediments (2.41%), respectively. Finally, the phylum Basidiomycota was found with percentage higher than 1% in L2 water (1.52%) and L5 sediments (3.76%).

Overall, the same phyla were retrieved in Antarctic samples, but with some crucial differences (Figure 13). First, Fungi_phy_Incertae_sedis showed an average percentage of around 26% and was about completely absent (0.04%) in water of LA, underlining a fungal community composition related mostly to known phyla. Also, in Antarctic samples Chytridiomycota was the most abundant phylum and ubiquitously distributed in all analyzed samples. The highest value was retrieved in water samples of LS (68.5%) and the lowest value was showed by the water sample of LT. The phylum Ascomycota in Antarctic lakes was retrieved with higher value than Arctic samples. It was found in all lakes with values comprised between 65.8% (water of LA) and 0.45% (water of LZ). Phyla Rozellomycota and Monoblepharomycota were retrieved with percentages higher than 1% only in LA sediments (40.3%) and LZ water (3.16%),

respectively. Finally, the phylum Basidiomycota was retrieved in all Antarctic samples with an average percentage of 4.5%. The highest value was found in water samples of LB (23.9%) and the lowest in water of LZ (0.06%).

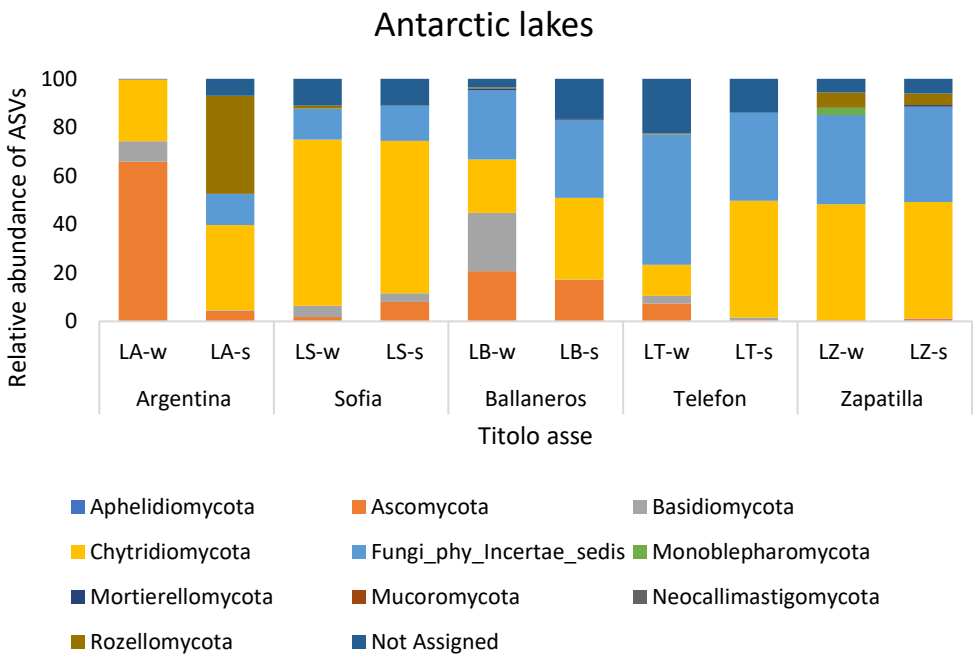


Figure 13. Fungal community structure at phylum level, in water and sediment samples from Arctic lakes.

Based on the retrieved phyla, statistical analyses of the variance were performed, and the relative abundance of the fungi that had high variance was selected (Ascomycota, Basidiomycota, Chytridiomycota, Rozellomycota and Fungi_phy_Incertae_sedis) for the construction of the PCA to identify groups of samples with similar community compositions (Figure 14).

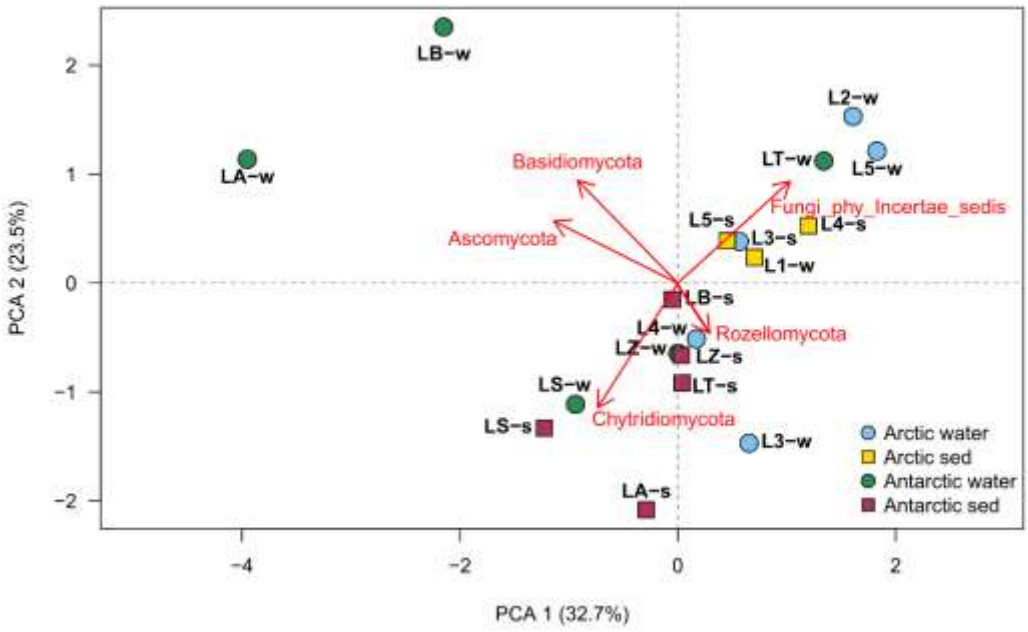


Figure 14. Principal component analysis obtained by retrieved phyla information parameters, made by factoextra R package.

The PCA showed also in this case a distinction between Arctic and Antarctic samples, in fact six of the eight Arctic samples clustered together and their separation was driven by Fungi_phy_Incertae_sedis. Two exceptions in the "Arctic cluster" were water from both lake L4 and lake L3 which resulted most related to the majority of Antarctic samples related to the presence of Chytridiomycota. Finally, two water samples of Antarctic region (lakes LA and LB) were completely separated by other group and noticeably related to the phyla Ascomycota and Basidiomycota. Retrieved phyla and environmental parameters were also used for the analyses of Spearman correlation (Table 12). Results showed a positive correlation between Aphelidiomycota, temperature and percentage of oxygen. Basidiomycota showed negative correlation with percentage of oxygen, and Chytridiomycota showed also negative correlation with the pH. In our samples also Ascomycota underlined a strong correlation ($p < 0.01$) with temperature, percentage of oxygen and pH.

Table 12. Table resuming all significant correlation retrieved among environmental parameters and retrieved phyla.

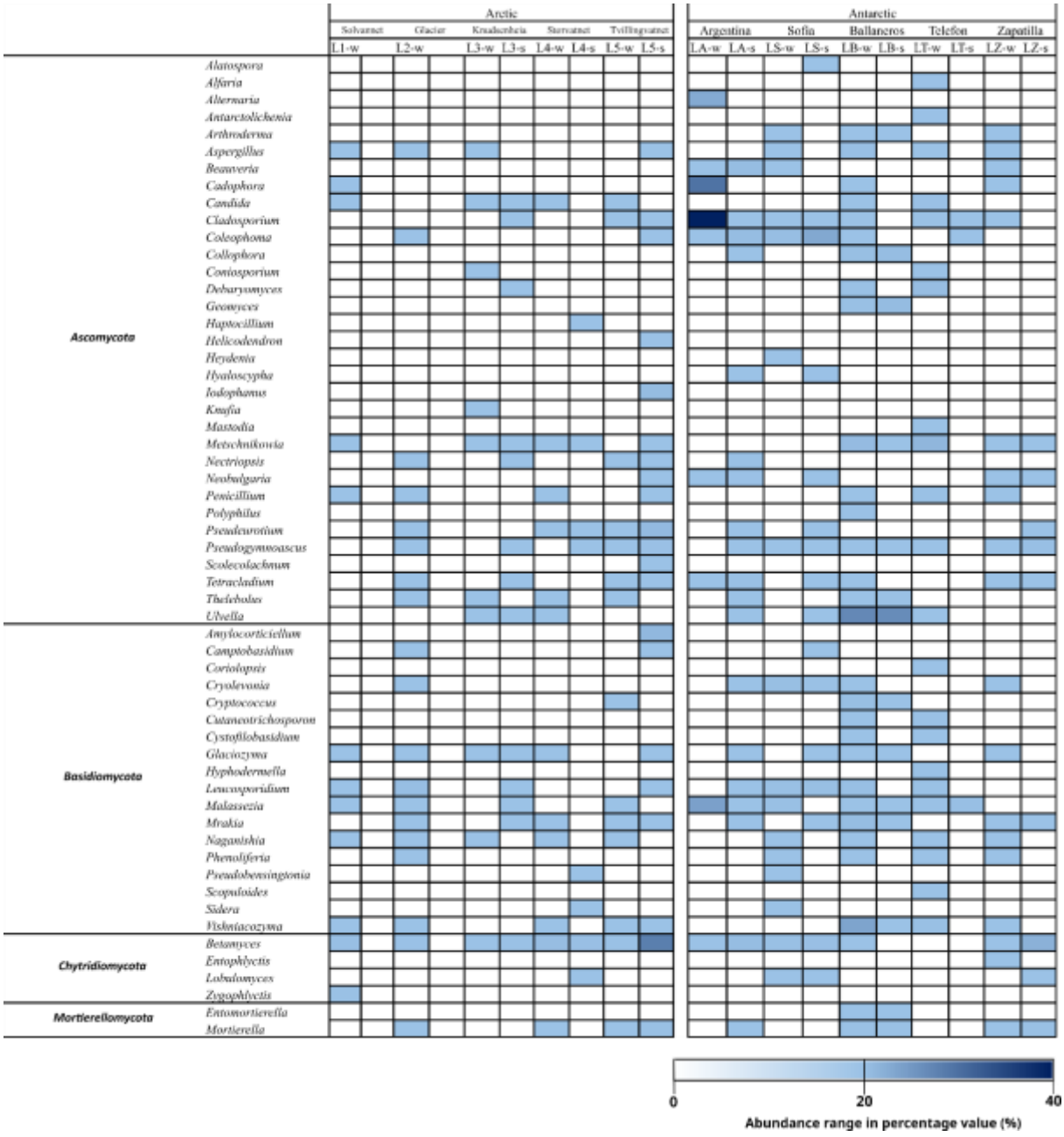
	Aphelidiomycota	Ascomycota	Basidiomycota	Chytridiomycota	Monoblepharomycota	Mortierellomycota	Mucoromycota	Neocallimastigomycota	Rozellomycota	Temperature	pH	O2 %	Cond (uS/cm)
Aphelidiomycota								0.0243	0.0020	0.0438		0.0273	
Ascomycota			0.0004							0.0044	0.0053	0.0002	
Basidiomycota		+										0.0152	
Chytridiomycota											0.0353		
Monoblepharomycota									0.0421				
Mortierellomycota													
Mucoromycota													
Neocallimastigomycota	+												
Rozellomycota	+				+								
Temperature	+	-									0.0010	0.0000	0.0106
pH		-		-						+		0.0000	
O2 %	+	-	-							+	+		0.0190
Cond (uS/cm)										+		+	

5.2.5 Fungal genera distribution in lakes' water and sediments

All retrieved genera were sorted and only the genera that showed, for at least one sample, a percentage above 1 % were outlined in Table 13. Overall, a total of 57 genera were detected. The majority of sequences classified at genus level were retrieved in Antarctic samples and, among all the analysed lakes, LA and LB (Antarctica) and L5 (Arctic) showed most of the genre-wide affiliated sequences. Regarding Argentina lake the genus *Alternaria* was retrieved at a percentage of 5.9% but only in water. Similarly, the genus *Cadophora* was retrieved principally in water of Argentina with a percentage of 19.5%, furthermore, very low percentages were retrieved in water of L1 (0.3%) and LB (0.1%). *Cladosporium* represented the most abundant genus retrieved in water of LA (39.5%), even if it was retrieved in almost all Antarctic samples with a total percentage of 40.3%. Instead, in Arctic samples this genus represented only 0.2%. The genus *Leucosporidium* showed a high abundance in LA water

sample (8.4%). In LB, *Ulvella* was the most abundant genus in both water and sediments samples (14.2 and 13.3%, respectively), followed by *Vishniacozyma* genus, which was retrieved with a percentage of 7.5% in water sample. Finally, the genus *Betamyces* was the most abundant in Arctic samples. Particularly, the highest abundance was retrieved in sediments of L5 (16.6 %). Even if with a very low percentage of abundance, this genus was retrieved also in almost all Antarctic samples. Generally, with metagenomics analyses it is very difficult to reach the genus level for fungi, and in our case particularly due to the extreme environments under consideration.

Table 13. Genera retrieved at a percentage above 1%.



5.2.6 Predicitive functional profiling of fungal communities

In total, a function was assigned to 103 ASVs (accounted for 1.7% of total ASVs). Genera with confidence level of "possible" were classified as "uncertained" and excluded from the functional analyses in this study. Retrieved function were assigned to saprotroph (57%), sathotroph (30.9%) and symbiotroph (12.1%) (Figure 15). For Arctic samples the functionality was assigned to a minor number of ASVs and pathotroph and saprotroph showed 3.03 and 6.7% in L2 and, 12.7 and 18.8% in lake L5 respectively. Particularly, it was underlined the saprotroph function for the ASV24 affiliated to the taxon *Betamyces* (L5). Interestingly, it was also observed the assignment of symbiotroph function to a great number of sequences retrieved in lake LA and related to the taxon *Cadophora.* In general, the prediction of function of our sequences showed the resolution to a very low percentage of ASVs, and this is probably due to the fact that samples as rare as that analysed in this study, have communities composed of a great number of organisms not identified and not yet studied. Spearman correlation was conducted using environmental factors and predicted function (Table 14). The results showed significant ($p < 0.05$) strict negative correlation between symbiotroph and conductibility and also negative correlation between saprotroph and pH.

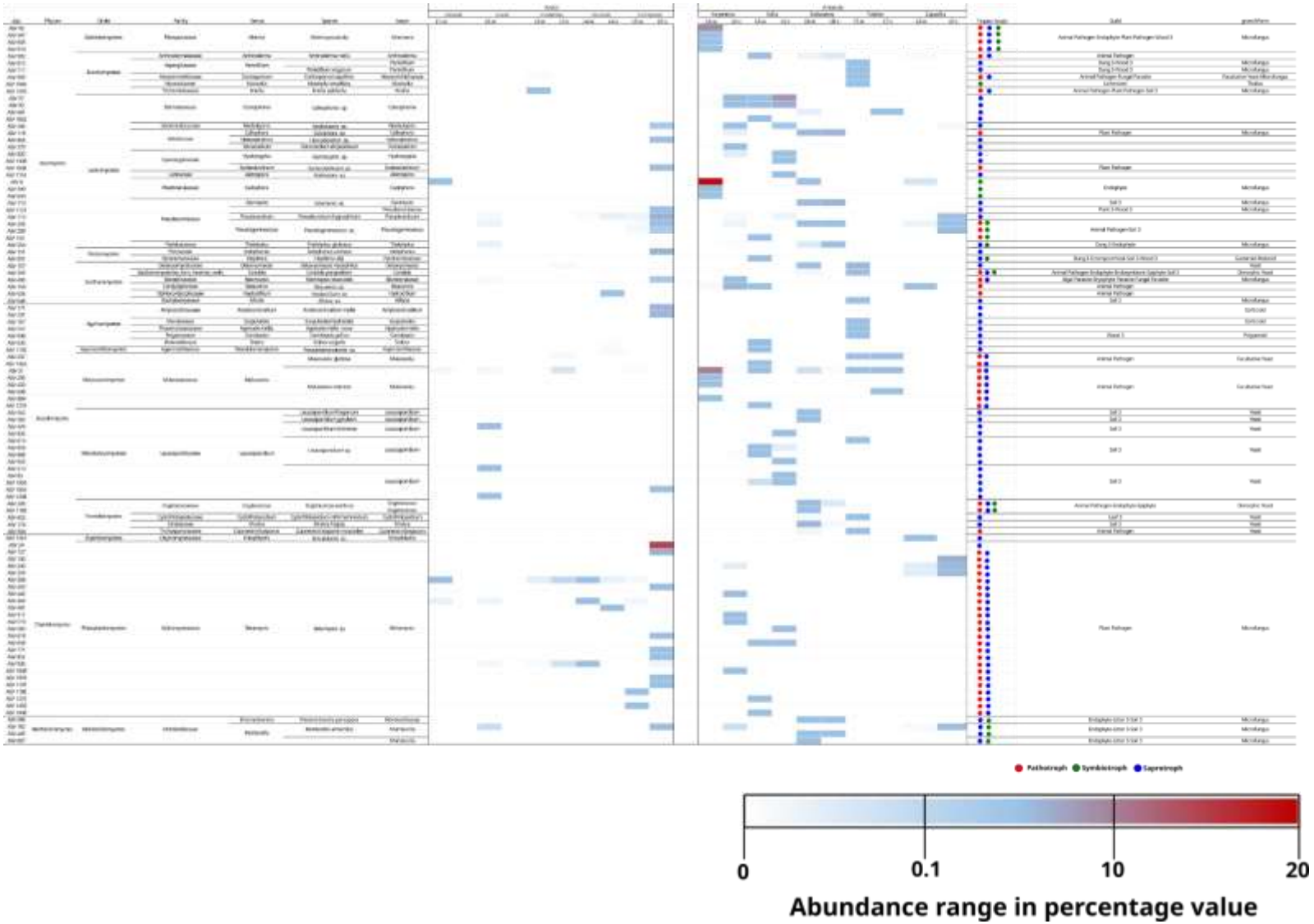


Figure 15. Guild of fungal species with an average relative abundance greater than 0.1% annotated byFUNGuild

Table 14. Table resuming all significant correlation retrieved among environmental parameters and predicted function.

	Pathotroph	Saprotroph	Symbiotroph	Temperature	pH	O2 %	Cond (uS/cm)
Pathotroph		0.0001	0.0039				
Saprotroph	+		0.0074		0.0498		
Symbiotroph	+	+					0.0001
Temperature					0.0010	0.0000	0.0106
pH		-		+		0.0000	
O2 %				+	+		0.0190
Cond (uS/cm)			-	+		+	

6 DISCUSSION

Microorganisms are the dominant life forms in Arctic and Antarctic regions. Amongst the groups of microorganisms present in these regions, fungi are one of the most present and better distributed in the various environments, and they play a crucial role in the micro and macro food web. Particularly fungi inhabiting polar lakes play a key role in biogeochemical cycles and the mineralization of organic matter, which are essential for the balance of micro- and macronutrients in lake systems. Many fungal species display multiple stress tolerance capabilities, surviving the combination of low temperatures, high salinity, pH variation, seasonally high UV radiation and low nutrient availability experienced in different polar lakes (Gonçalves et al. 2012; Ogaki et al. 2019; Rosa et al. 2019). However, despite their outstanding importance, the availability of studies of fungal diversity in polar lakes has increased only in recent years, but still remains limited. Most previous studies of polar lake fungal communities have used traditional culture-dependent methods (Ellis-Evans, 1996; Brunati et al. 2009; Vaz et al. 2011; Gonçalves et al. 2012; Ogaki et al. 2020a, b; Connel et al., 2018, Grum Grzhimaylo et al., 2018; de Souza et al., 2022b) which, as with all microbial groups, do not reveal the full complexity of the resident fungal diversity. Recent applications of metabarcoding approaches have focused especially on sediment of lakes of maritime Antarctica (Ogaki et al., 2021a; de Souza et al., 2022a; Rosa et al., 2022a) and there is only one study on water samples from lakes of continental Antarctica (Rojas-Jimenez et al, 2017). Very few metagenomic studies were reported also for Arctic lakes (Comeau et al., 2016; Zhang et al., 2016; Perini et al., 2019) supporting our findings that about 50% of ASV obtained for Artic samples were not assigned to a fungal phylum, providing evidence that there are many currently unknown fungal taxa in these Arctic environments.

To date, this thesis work represents the first study of fungal communities in water and sediment of both, Arctic and Antarctic, lakes.

As shown by our results of cultivation analysis, a low fungal abundance was obtained for all water and sediment samples for all polar lakes. In water samples of Arctic lakes, values of abundances

ranged from 2.5 CFU/100 mL to 81 CFU/100 mL, where in particular, Lake Solvannet (L1) harbored the highest number of cultivable fungi (up to 81 CFU/100 mL), while Lake Knudsenheia (L3), harbored the lowest number (up to 3.75 CFU/100 mL). These results could be related to the location of these lakes and to the influence of the surrounding environments. In fact, Lake Solvannet, located in to the scientific town of Ny-Ålesund, is frequented by numerous birds during the summer, and this could be a source of contaminant, but specially of organic matter which makes this environment thriving for fungi life. On the contrary, Lake Knudsenheia is located far away from the built-up area and close to the seacoast, thus being affected by sea water as suggested by its values of water conducibility (higher salinity) (Table 2), making this place less suitable for fungal proliferation. Very low number of isolates were obtained for lakes sediments of Artic, according to result reported by Grum Grzhimaylo et al. (2016), from the bottom sediment in various samples of Lake Kislo-Sladkoe (Karelian coast of Kandalaksha Bay, Canada).

Regarding the lakes analyzed in the Antarctic region, in water samples the highest fungal count was obtained in Lake Ballaneros (LB) (up to 42 CFU/100mL), while no growth was obtained in water samples collected from Lake Extremadura (LE) which is completely agrees with the results of ITS PCR amplification control, from the total DNA extracted from water sample that failed. The fungal abundance in the other Antarctic lakes was between 0.75 CFU/100 mL and 13 CFU/100 mL. These values are almost comparable with resulted obtained by Gonçalves et al. (2012) in a study of diversity of culturable fungi in water samples, conducted on 6 lakes from Deception Island and King George Islands.

To further investigate the fungal diversity in polar lakes, a NGS metagenome (metabarcoding) approach was carried out. The total fungal community detected through metabarcoding shows, within the analysed lakes, comparable values of diversity (Table 11), underlining no overall difference between Arctic and Antarctic lakes, while a small difference is present between water and sediments, showing a slightly greater diversity for sediment than for water. Comparable results were obtained by Perini et al. (2019), which calculated diversity in waters one lake from Ny-Ålesund with a Shannon

index H′ = 3.27, corresponding to the mean value obtained in this study (H′=3.3), while greater values (comprised between H′=3.83 and 5.24) were obtained by Zhang et al. (2016).

The diversity data of fungal sequence assemblages detected in the sediments of Antarctic lakes Argentina, Ballaneros, Sofia, Telefon, and Zapatilla studied here were greater than those reported in other culture-based studies (Gonçalves et al., 2012; Ogaki et al., 2020a; de Souza et al., 2022b). Overall, the diversity data obtained in this study by DNA metabarcoding were comparable with those reported by Ogaki et al. (2021a), de Souza et al. (2022a), Rosa et al. (2022a), and Gonçalves et al. (2022). However, results obtained by de Souza et al. (2022a) in sediments of Lake Soto, located on Deception Island (Antarctic Peninsula), where three lakes examined here (Ballaneros, Telefon and Zapatilla) are situated, showed lower diversity indices (Fischer =10.27).

Although comparable diversity was observed between the studied lakes in the two regions (Arctic and Antarctic), a considerable difference was observed in terms of composition. In fact, out of 5980 ASVs only 102 ASVs (1.7%) were shared between Arctic and Antarctic samples. This result suggests that fungal distribution varies between the lakes in the two Polar regions and each counterpart hosts specific fungal taxa. Not only geographical distance but also physical-chemical parameters which separate lakes of the two regions (as shown in Figure 8), could contribute to shaping the composition of the fungal community in lakes belonging to the two different regions.

For Arctic lakes, unknown fungi dominated the sequence assemblages, with almost half of the obtained ASVs being assigned to Fungi_phy_Incertae_sedis. This assignation suggests the dominance of possibly undescribed fungi, or that these taxa provide examples of sequences not currently included in publicly accessible databases. The problem arises from the scarcity of metagenome studies of fungal communities in these polar ecosystems, in particular for Arctic lakes. This fact is also corroborated by the result obtained with the PCA of each fungal group for each sample (Figure 14), where almost all fungal assemblages in Arctic water and sediment correlated with the group of Fungi_phy_Incertae_sedis.

The most represented identified phyla were, in order, Chytridiomycota (24.82%), Rozellomycota (4.16%), Ascomycota (0.98%), Basidiomycota (0.8%), which were commonly reported by Comeau et al. (2016), Zhang et al. (2016) and Perini et al. (2019) in Arctic lakes. Less frequent, instead, Monoblepharomycota (0.36%), Aphelidiomycota (0.25%), Mortierellomycota (0.1%), Neocallimastigomycota (0.01%) and Mucoromycota (0.002%) which were never reported in Arctic lakes, but (excluding the Phylum Neocallimastigomycota) were previously detected in Antarctic lakes (Ogaki et al., 2021a; Rosa et al., 2022a; de Souza et al., 2022a; Gonçalvez et al., 2022).

In Antarctic samples, the percentage of ASVs assigned to unknown fungi was lower (average of 26.70%) than the ASVs obtained from Arctic samples, probably due to the higher number of studies of fungal communities in Antarctic lacustrine systems that increased considerably in recent years. Chytridiomycota results as the most represented phylum (40.41%), followed in the order by Ascomycota (12.74%), Rozellomycota (5.33%), Basidiomycota (4.54%) Monoblepharomycota (0.36%), Mortierellomycota (0.21%) and Aphelidiomycota (0.002%).

Different studies showed that members of the phyla Chytridiomycota and Cryptomycota (Rozellomycota) dominated the fungal community composition in European freshwater lakes (Monchy et al., 2011; Wurzbacher et al., 2016; Lepere et al., 2006). Similar results have also been obtained in marine and polar freshwater environments (Zhang et al., 2015; Zhang et al., 2016; Comeau et al., 2016), and the recent use of DNA metabarcoding approaches has revealed the presence of Chytridiomycota and Rozellomycota assigned sequences also in Antarctica, with reports from soil (Rosa et al., 2020a), air (Rosa et al., 2021a), mosses (Rosa et al., 2021b), permafrost (da Silva et al., 2022), marine sediments (Ogaki et al., 2021b), snow (Rosa et al., 2022b) and, recently, in lake sediments (Ogaki et al., 2021a; Rosa et al., 2022a; de Souza et al., 2022a). Particularly the study of Rojas-Jimenez et al. (2017) detected sequences of Chytridiomycota together with Cryptomycota (Rozellomycota) dominating the fungal communities in waters of ice-covered lakes of the McMurdo Dry Valleys (continental Antarctica). The phyla Rozellomycota and Chytridiomycota can be particularly common in anoxic environments (Ogaki et al., 2021a), but their peculiarity is that these

fungal phyla have some physiological advantages for inhabiting aquatic ecosystems, including their mobility and capacity to parasitize numerous phytoplankton species such as diatoms, green algae, dinoflagellates and cyanobacteria (Kagami et al., 2007; Ishida et al., 2015). Furthermore, the Chytridiomycota are implicated in a variety of ecological processes, such as the transfer of organic matter from phytoplankton into zooplankton via saprophytic and parasitic activity (Kagami, 2014). In particular, in our study the phylum of Chytridiomycota was reported as dominant in almost all samples, and its role as parasites of freshwater algae and zooplankton is well known (Redfield et al., 1979; Sparrow, 1943; Sime-Ngado, 2012) and taxa in this group are thought to mediate the transfer of organic matter from phytoplankton into zooplankton via saprophytic and parasitic activity described as the "mycoloop" (Kagami, 2014). Freshwater chytrids are also known as parasites of larger animals such as amphibians (Berger et al., 2016).

The phylum Ascomycota was the most represented in water samples of Lake Argentina (LA) with a percentage of 65.87%. This dominance could be related to the origin of this lake, which derives from the ice-melting of a close glacier. In a recent DNA metabarcoding study (Rosa et al., 2022a), it was reported that Ascomycota represents the dominant phylum in Antarctic snow. So, by the water supply from the glacier, this phylum could enrich the fungal composition of the lake.

In this study the infrequent phylum Neocallimastigomycota was found exclusively in Arctic lakes, in water of Solvannet (L1) and in sediments of Knudsenheia (L3), Storvatnet (L4) and Tvillingvatnet (L5). This phylum was never reported in studies of polar lakes. The members of Neocallimastigomycota are anaerobic-flagellate fungi residing in the rumen and alimentary tract of larger mammalian and some reptilian, marsupial and avian herbivores, where they play an important role in the degradation of plant material (Gruninger et al., 2014; Hanafy et al., 2021). The detection of this phylum in Arctic lakes analysed in this study could be due to the presence of birds or moose which visit these lakes. Neocallimastigomycota DNA was already reported in soil (Lockhart et al., 2006), and estuarine sediments (Devon & Martiny, 2011), and this is consistent with their ability to disperse efficiently between hosts. However, the failure of most efforts to isolate anaerobic fungi

from non-gut habitats suggests that it is only the aerotolerant propagules of these fungi that remain viable in the wider environment (Gruninger et al., 2014).

At the genus level, the most represented genera found were *Alternaria* (exclusively from Antarctica), *Cladosporium*, *Cadophora*, and *Ulvella* (Ascomycota), *Leucosporidium* and *Vishniacozyma* (Basidiomycota), and *Betamyces* (Chytridiomycota), most of which have previously been reported from different environment in the Polar regions. *Alternaria*, *Cladosporium* and *Cadophora* are melanized ascomycetous fungi distributed worldwide and occupy various ecological niches. They are known to be able to resist harsh environmental conditions such as high temperatures, scarcity of water, and high UV radiation (De Leo et al., 2022). Most species of these genus are plant pathogens or endophytes (Walsh et al. 2018; Zabouri et al., 2021), wood destroyers (Travadon et al., 2015), and soil inhabitants (Domsch et al., 2007). Some species of the genus *Cadophora* and *Cladosporium* are psychrotrophs (Iliushin et al., 2020; Brück et al., 2022) and the presence of these genera are well documented both in Antarctica (Blanchette et al., 2004, Arenz & Blanchette 2009; Ogaki et al., 2019) and in the Arctic (Kirtsideli et al., 2014; Zhang et al., 2016; Bubnova & Nikitin 2017; Perini et al., 2019). The genus *Leucosporidium* was described during investigation of Antarctic heterobasidiomycetes (Fell et al., 1969). It comprises 12 species, mostly phychrotolerant or psychrophilic, which occur in plant materials, soils, and marine environments of high and moderate latitudes (Yurkov et al., 2012; Laich et al., 2014; de García et al., 2015; Crous et al., 2019). Members of the genus *Leucosporidium* are biotechnologically important microorganisms as sources of low temperature extracellular enzymes and due to their ability to biodegrade phenolic compounds (de García et al., 2015).

Vishniacozyma is a cosmopolitan genus, and has been reported from cold environments around the world, including subglacial ice samples from Svalbard Island and from glaciers in the High Canadian Arctic (Perini et al., 2019); from soil and wood in the Ross Sea region, from soil of South Victoria Land (Arenz et al., 2006; Connell et al., 2008), from Antarctica soil samples of Victoria Land, Lichen Valley, Vestfold Hills, Davis Base, and from the Skarvsnes ice-free area (Montes et al. 1999; Thomas-

Hall et al. 2002; Vaz et al. 2011, Tsuji et al. 2013). The genus *Betamyces* (Chytridiomycota) was retrieved in freshwater ecosystem in Ny-Ålesund (Arctic) (Zhang et al., 2016) and in sediment of Antarctic lakes, were *Betamyces* sp. dominated the assemblages (Gonçalves et al., 2022). The genus includes only one known species, *Betamyces americae-meridionalis*, which was isolated from pollen baits at the Paraná a River, Buenos Aires, Argentina and in soil in Costa Rica (Letcher et al., 2012). The literature about the genus *Ulvella* is very scarce. There is only one species, *U. chlorospila* synonym *Pyrenula chlorospila* Arnold. The genus *Pyrenula* is a group of crustose lichens typically growing on smooth, shaded bark. It comprises 745 named species with worldwide distribution, most represented in the tropics and Europe (Aptroot, 2012), but never reported from polar environments. In our study, we deeply investigate fungal communities by the use of functional prediction, and the results consist of the Arctic and Antarctic fungi displaying different ecological roles as saprophytes, mutualists, symbionts, and/or parasites. Saprophytic fungi dominated the assemblages detected in water and sediment of Arctic and Antarctic lakes examined, followed by plant and animal pathogens and symbionts and the same situation was observed in sediment of lakes on Vega Island, Elephant Island, Deception Island, Jame Ross Island, and Trinity Peninsula (Ogaki et al., 2021a; de Souza et al., 2022a; Rosa et al., 2022a; Gonçalvez et al., 2022), all in maritime Antarctica. The same functional ecological profiles have been reported in metabarcoding studies of different Antarctic habitats, such as air and/or snow (Rosa et al. 2020b, 2021a), soil (Rosa et al. 2020a), freshwater (de Souza et al. 2021) and rock surfaces (de Menezes et al. 2021). According to Schütte et al. (2019), fungi present in polar environments display the capability to degrade organic matter at low temperatures, thereby releasing compounds containing carbon, nitrogen, and other elements to other organisms. The dominance of saprophytic fungi in water and sediment of the examined Arctic and Arctic lakes might indicate, as suggest by de Souza et al., (2022a) the presence in these environments of a complex saprophytic fungal community that plays a vital role in the decomposition of organic matter under extreme conditions.

The current study was also focused on fungal resistance to heavy metals, and we obtained a total of 18 fungal strains for their ability to grow in presence of HMs concentrations up to 5000 ppm (Fe and Cu) and 500 ppm (Hg). By ITS analysis, they were assigned to the following taxa: *Pseuderotium* sp., *Rhodotorula* sp., *Glaciozyma* antarctica, *G. watsonii, G. martini Cadophora fastigiata, Helotiales* sp., *Holtermanniella wattica, Mrakia robertii,* and *Cryptococcus* sp.

The genus *Pseuderotium* was previously reported in Arctic and Antarctic different environments and substrata, such as lakes (Gonçalves et al., 2012; Zhang et al., 2016; Perini et al., 2019; Ogaki et al., 2020a), active layer of the ice-free oases in continental Antarctica (Kochkina et al., 2014), sponges (Henríquez et al., 2014), and the soil (Arenz & Blanchette 2009). The species *P. hygrophilum* was isolated from soil impacted by human activities in the Bellingshausen Russian station (King George Island), and may be involved in degradation of soil contaminants (Kochkina et al., 2018).

Based on the literature, *Cryptococcus*, *Rhodotorula*, and *Mrakia* have been found in many cold climates throughout the world and are the main yeasts living in different Arctic and Antarctic environments, including lakes (Zhang et al., 2016; Buzzini et al., 2012; Carrasco et al., 2012; de Souza et al., 2022b). The tolerance of these yeasts to different HMs is already know. In fact, species belonging to the genera *Cryptococcus*, *Rodothorula* and *Mrakia* isolated from Antarctic soils were previously reported to be able to tolerate HMs such as chrome, cadmium and copper (Fernandez et al., 2017) in the concentration of 1 mM. In a study of Sing et al. (2013) a psychrotolerant yeast of genus *Cryptococcus* isolated from sediments of the Central Indian Basin demonstrated considerable growth in the presence of 100 mg/L of heavy metal salts i.e. zinc, copper, lead and cadmium. The species *Mrakia robertii*, assigned to our Fe tolerant strain Y-AZA-1, was described by Thomas-Hall et al. (2010). The species is psychrophilic, firstly isolated from lichen and soil in Vestvold Hills (Antarctica) and in a glacier from Italian Alps. The species was also found in polar lakes in King George Island (Antarctica) (Ogaki et al., 2020a). The isolate Y-AAA-2 was identified as *Cadophora fastigiata*. Species in the genus *Cadophora* have been identified in many extreme environments, including polar areas (Held et al., 2017). The genus was also isolated from heavy metal contaminated

sites (Gorfer et al., 2009) and copper treated wood (Karunasekera et al., 2019). The species *Cadophora fastigiata*, in particular, was isolated from iron mine (Rusman et al., 2015, Held et al., 2020) where presence of high levels of heavy metals was registered, so the presence of this species in such extreme environments indicates an adaptation to withstand high heavy metals, cold temperature and other stress factors.

Three yeast strains tolerant to high concentrations of Hg were assigned to *Holtermanniella wattica*. The species was previously isolated from soil and lake in King George Island (Carrasco et al., 2012; de Souza et al., 2022b). The genus was established by Libkind, Wuczkowski, Turchetti and Boekhout (Wuczkowski et al., 2011), to accommodate the anamorphic *Cryptococcus* species that were included phylogenetically in the Holtermannia clade, and the species *Holtermanniella wattica* (basionym: *Cryptococcus watticus*) was also described. In a study by Fernandez et al. (2017) a strain of *Cryptococcus watticus* showed tolerance to HM such as Cr and Cu.

The new genus *Glaciozyma* was proposed by Turchetti et al. (2011), which provided also the description of the species *Glaciozyma martinii* and *Glaciozyma watsonii*, and the re-classification of *Leucosporidium antarcticum* as *Glaciozyma antarctica*.

Glaciozyma species are obligate psychrophiles, with a maximum growth temperature below 20 °C (Turchetti et al., 2011). They are frequently found in persistently cold habitats such as Alpine and Apennine glaciers (Price et al., 2009; Wang et al., 2015), sea water, soil and lake sediment in Antarctica and Greenland glacial ice (Connel et al., 2008; Ogaki et al., 2020a), glaciers and associated aquatic environments in the Arctic (Perini et al., 2019). Foong et al. (2015) conducted a survey on the metal preference of the strain *G. antarctica* PI12, using the integration of multiple sequence-based bioinformatics approaches. The results showed that a quarter of its proteome was found to be metal-enriched, with the majority of these proteins using essential metals as cofactors. Interestingly, the presence of heavy metal-binding proteins (e.g.: cadmium, mercury and lead) was also recorded.

Other two fungal strains isolated from the enrichment with Fe 1000 ppm, Y-ACA2 and Y-ATA3 were assigned to the genus *Pseudogymnoascus* sp. and *Exophiala* sp., respectively. The genus

Pseudogymnoascus (syn. *Geomyces*) includes many species common in cold and polar ecosystems, occurring in alpine, temperate, Arctic and Antarctic ecosystems (Mercantini et al. 1989; Rice & Currah 2006; Lorch et al., 2013; Minnis & Lindner 2013). In Antarctica, representatives of *Pseudogymnoascus* have been reported in soils (Mercantini et al. 1989; Arenz and Blanchette 2011; Krishnan et al., 2011; Gomes et al., 2018), associated with plants (Tosi et al. 2002; Rosa et al. 2010; Carvalho et al. 2020), macroalgae (Loque et al. 2010), lichens (Santiago et al. 2015) and also in sediment of freshwater lakes (Gonçalves et al. 2012).

Exophiala sp. is a ubiquitous dematiaceous genus in the order Chaetotyriales known for the ability to withstand different hostile environments. In particular, the genus has frequently been isolated from anthropogenic polluted sites: the ability of *Exophiala* species such as *E. oligosperma*, *E. xenobiotica*, and *E. mesophila* to metabolize alkylbenzenes as the sole source of carbon and energy may explain their success in colonizing such environments (Blasi et al., 2016; Isola et al., 2013). In addition, the genus has been proven to be potential causative agent of human or animal disorders (de Hoog et al., 2006). In Antarctic, species of the genus *Exophiala* were isolated from endolithic ecosystems (Coleine et al., 2019), soil (Arenz et al., 2006), permafrost (Kochkina et al., 2012), seawater (Gonçalves et al., 2017) and lake freshwater (Vaz et al., 2011).

7 CONCLUSIONS

Still today, the studies carried out on the fungal communities of lake ecosystems in Arctic and Antarctic lakes are decidedly few, the majority of them have been based primarily on traditional culture-dependent approaches, thus underlining a still large gap in the understanding of these sensitive environments. In fact, this study is the first to compare fungal communities in water and sediment of Arctic and Antarctic lakes. The results obtained show clearly distinct communities between the analyzed environments, probably due to the different environmental factors and limnological differences between the analyzed lakes, however showing some common threads. In particular, the most frequently found phyla are generally ubiquitous, even if for the first time the presence of Neocallimastigomycota is reported in Arctic samples. Furthermore, our results also highlight the dominance of a saprophytic fungal community which as widely reported as plays a vital role in the decomposition of organic matter under these extreme conditions. The study of the fungal response to heavy metals highlighted numerous fungal genera already widely recognized as HMs tolerant, thus showing that fungi in these areas also have a significant role in environmental decontamination. Furthermore, the excellent results in response to heavy metal tolerance of the Y-AZA2 strain (affiliated to *Cryptococcus* sp.) are still under investigation for the analyses of its transcriptome, which will then be studied in search of metabolic pathways and proteins related to metal resistance. Therefore, even if numerous studies will still be necessary, this work is the first in the comparative study of such extreme environments and highlights numerous focal points for future works.

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Acknowledgments

My special thanks to:

Dr. Maria Papale, researcher at the Institute of Polar Sciences, National Research Council (CNR-ISP) of Messina, for her help in molecular and bioinformatics investigations, but especially for precious teachings, professional and personal support she provided me during my PhD career, encouraging me to do my best.

Dr. Maurizio Azzaro and Dr. Angelina Lo Giudice, respectively responsible and researcher at Institute of Polar Sciences, National Research Council (CNR-ISP) of Messina, for giving me the chance to work in the labs of the CNR-ISP of Messina to do part of my thesis work and for the sympathy they showed to me.