Akumulacja, mobilność i biodostępność radioizotopów oraz ich wpływ na wybrane elementy ekosystemu supraglacjalnego

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Accumulation, mobility and bioavailability of radioisotopes and their impact on selected components of supraglacial ecosystem

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PhD Thesis

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Contents

Streszczenie

Antropogeniczny wpływ na różne poziomy organizacji ekosystemów znacznie wzrósł od drugiej wojny światowej, szczególnie na obszarach wysoce uprzemysłowionych. Nawet odległe regiony górskie i polarne nie są już wolne od zanieczyszczeń, a dziedzictwo naszych działań w postaci zanieczyszczeń zostało rozprzestrzenione na całym świecie. Jednym ze stresorów wysokiego ryzyka są radioizotopy, które stały się szczególnym zagrożeniem w wyniku działań wojskowych i awarii elektrowni jądrowych. Radioizotopy wytworzone przez ludzi, zwane radioizotopami sztucznymi, osiągnęły szczyt depozycji w latach 60. XX wieku. Z powodu długiego okresu półtrwania niektórych z nich, utrzymują się one w ekosystemach.

Ciemny biogeniczny osad na powierzchni lodowców skutecznie gromadzi atmosferyczne zanieczyszczenia, w tym radioizotopy. Ten osad, zwany kriokonitem, jest istotnym komponentem funkcjonowania ekosystemów lodowych i ekosystemów przylegających, ponieważ dostarcza składniki odżywcze na lodowcach oraz na ich przedpolach. Natomiast, ze względu na ciemny kolor przyspiesza topnienie lodowców. Stężenie radioizotopów w kriokonicie jest wyższe niż w otaczających siedliskach. Kluczowe są zatem działania na rzecz zrozumienia jaki wpływ mogą mieć podwyższone stężenia radioizotopów na ekosystemy lodowcowe oraz co kształtuje rozmieszczenie i stężenie radioizotopów. Głównym celem tej pracy jest zrozumienie, czy podwyższone stężenia radioizotopów mogą zagrozić organizmom zamieszkującym lodowce i otaczające ekosystemy.

Wyniki uzyskane w ramach tej pracy zapewniają kompleksowy przegląd rozmieszczenia przestrzennego, bioakumulacji i potencjalnych zagrożeń promieniowania jonizującego na organizmy lodowcowe poprzez analizę 22 lodowców alpejskich. Badania te są jednymi z niewielu, które próbują odpowiedzieć na pytanie, czy antropogeniczna radioaktywność wpływa również na organizmy poza miejscami katastrof lub miejscami testowania broni jądrowej i jako pierwsze określają relacje organizmów z radioizotopami na lodowcach.

Wyniki pierwszego rozdziału wskazały na pozytywny związek między stężeniami aktywności ^{137}Cs i ^{210}Pb a zawartością materii organicznej. Ponadto ^{210}Pb był również dodatnio związany ze stężeniem chlorofilu, co sugeruje wpływ społeczności fotoutotroficznych na bioakumulację. Analiza mobilności ujawniła, że ^{137}Cs jest w 72% związany z minerałami, natomiast ^{210}Pb jest bardziej mobilny i związany z kompleksami

organicznymi. Cez-137 jest częściowo związany z materią organiczną, ale mocno związany z powierzchnią minerałów, może zatem oddziaływać na organizmy, które budują biofilm na ziarnach mineralnych. Zaobserwowano u szczytowych konsumentów akumulację radioizotopów ^{137}Cs, ^{210}Pb i $^{239+240}$Pu. Są to jednak wartości dużo niższe niż w obszarach silnie zanieczyszczonych, takich jak strefa wykluczenia w Czarnobylu. Podsumowując, wyniki wskazują, że radionuklidy wykazują różne relacje przestrzenne z organizmami i prawdopodobnie mają inną drogę rozprzestrzeniania się podczas topnienia lodowca.

W drugim rozdziale, analiza ^{137}Cs, ^{210}Pb i ^{241}Am między lodowcami ujawniła, że cechy lodowca (powierzchnia, wysokość i ilość materii organicznej) odgrywają bardziej znaczącą rolę w akumulacji radioizotopów niż czynniki geograficzne i opady. Oprócz zawartości materii organicznej w kriokonicie i wysokości (znaczącej dla ^{210}Pb), powierzchnia lodowca jest najważniejszym predyktorem stężeń aktywności radioizotopów na alpejskich lodowcach. Stężenie aktywności naturalnych i antropogenicznych radioizotopów są wyższe na małych lodowcach niż na dużych. Sugeruje to, że znaczna ich część jest wiązana z kriokonitem podczas topnienia lodowców.

Najważniejszym wynikiem trzeciego rozdziału jest negatywny wpływ podwyższonej radioaktywności wskutek akumulacji ^{137}Cs i ^{210}Pb na mikroorganizmy w otworach kriokonitowych. W przypadku bakterii efekt jest podobny dla obu radionuklidów, natomiast w przypadku zgrupowań eukariotycznych ^{210}Pb ma silniejszy wpływ na zmniejszenie bogactwa gatunkowego. Wyniki te są poparte oceną ryzyka w poprzednich rozdziałach, z których wynika, że ^{210}Pb jest silniej związany z martwą materią organiczną i organizmami, natomiast ^{137}Cs z frakcjami mineralnymi.

Teza zawiera pierwsze dowody na to, że podwyższone stężenia radioizotopów na lodowców są efektem bioakumulacji przez organizmy lodowcowe co może zagrażać bioróżnorodności i funkcjonowaniu tego ekosystemu. Jest to ważne, ponieważ zgodnie z przewidywaniami, małe alpejskie lodowce znikną w ciągu 50 lat, uwalniając radioizotopy. Zakładając ten scenariusz, możemy być świadkami intensywnej presji abiotycznej na ekosystemy związane z lodowcami. Systematyczne i skoordynowane monitorowanie zanieczyszczeń uwalnianych przez lodowce jest kluczem do zrozumienia tego problemu w skali globalnej.

Abstract

Anthropogenic impact on the ecosystem components at different levels of organisation has significantly increased since the Second World War, especially in highly industrialised areas. However, even remote polar and mountain regions are not contaminant-free anymore, and the legacy of our actions in the form of pollutants has spread over the globe. One of the high-risk stressors are radioisotopes, which became a particular threat as a result of military actions and nuclear power plant accidents. Radioisotopes formed by humans, called artificial radioisotopes, peaked in deposition in the 1960s. However, due to the long half-life of some of them, they persist in the global ecosystem.

Dark biogenic sediment on the surface of glaciers worldwide effectively accumulates atmospheric-delivered pollutants, including radioisotopes. This sediment, called cryoconite, is important in the functioning of the supraglacial ecosystems and glacier-adjacent ecosystems, as it can provide nutrients for biological succession after glacier retreat. On the other hand, due to its dark colour promotes glaciers melting. The concentration of radioisotopes in cryoconite is higher than in surrounding habitats. Therefore, all actions towards understanding what impact elevated concentrations of radioisotopes may have on glacial ecosystems and what shape radioisotope distribution and concentration are crucial. The primary goal of this thesis is to understand whether elevated concentrations of radioisotopes may threaten organisms inhabiting glaciers and surrounding ecosystems.

The results obtained in this study provide a comprehensive overview of the spatial distribution, bioaccumulation and potential threats of increased levels of ionising radiation on glaciers by analysing 22 glaciers covering the most glaciated areas of the Alps. This research is one of the few that attempts to answer the question of whether anthropogenic radioactivity also affects organisms outside nuclear disaster sites or nuclear weapons testing sites and first investigates radionuclide-organisms relations on glaciers.

The results of the first chapter revealed a positive association between ^{137}Cs and ^{210}Pb activity concentrations and organic matter content. In addition, ^{210}Pb was also positively related to chlorophyll concentration, suggesting an influence of the photoautotrophic communities in the bioaccumulation. The mobility analysis has revealed that ^{137}Cs are firmly bound to minerals in 72%, while ^{210}Pb is more mobile with

a high fraction bound to organic-metallic complexes. ^{137}Cs is only partially associated with organic matter. However, as it is firmly bound to the outer layers of minerals, it can interact with microbial communities that build biofilms on mineral grains, which seems an important fraction in cryoconite. The activity concentrations observed in the top consumers were detected but relatively low for ^{137}Cs, ^{210}Pb and $^{239+240}$Pu, while not detectable for ^{238}Pu being orders of magnitude lower than those found in contaminated areas like the Chernobyl exclusion zone. Overall, the results indicate that both radionuclides show different spatial relations with organisms and likely have a different fate during glacier melting.

Interglacial analysis of ^{137}Cs, ^{210}Pb and ^{241}Am in the second chapter has revealed that glacier features (surface area, altitude and the amount of organic matter in the cryoconite) play a more significant role in the accumulation of radioisotopes than geographic factors. Apart from the organic matter content in cryoconite and elevation (significant for ^{210}Pb), the glacier's surface area emerges as the most critical predictor of radioisotope activity concentrations on Alpine glaciers. The activity concentration of natural and anthropogenic radioisotopes increases as the glacier's surface area decreases. This suggests that a significant proportion of radioactivity is bound to the supraglacial sediment during glaciers melting.

The most important outcome of the third chapter is that microbial communities in cryoconite holes on the surface of glaciers are influenced by environmental radioactivity due to elevated activity concentrations of fallout ^{137}Cs and ^{210}Pb. In the case of bacteria, the effect is similar for both nuclides, while in the case of eukaryotic communities, ^{210}Pb has a stronger effect for decreasing species richness. These results are supported by the risk assessment in the previous chapters, where ^{210}Pb is more strongly associated with organic matter and organisms itself, while ^{137}Cs with mineral fractions.

Overall, the thesis provides the first evidence that elevated concentrations of radioisotopes on glaciers are an effect of bioaccumulation by surface glacier biota and can threaten biodiversity and ecosystem function. This might be important, as the small glaciers in the Alps are predicted to disappear within the next 50 years, releasing stored radioisotopes. If so, we could witness intense abiotic pressure on glacier and glacier-adjacent ecosystems. A systematic, coordinated monitoring of pollutants released by glaciers can be a key to understanding this problem globally.

Introduction

Earth has experienced strong pressure from pollutants released by human into the environment during the 20th century. Highly industrialized areas were most vulnerable (Crippa et al., 2021), but the legacy of our activities has affected all ecosystems on our planet (Häder et al., 2020; Pittino, Buda, et al., 2023), even those far from permanent human habitation, such as high mountains, polar regions (Buda, Łokas, et al., 2020; Mietelski et al., 2008; Pourchet et al., 2003) or the depths of the oceans (Dasgupta et al., 2018).

Radioisotopes, also called radionuclides, became a particular threat in the 20th century. The progress in research on the structure of the atom facilitated development of nuclear technology, at the same time formed a new harmful pollutants such as artificial radionuclides. Radioisotopes are atoms with unstable nuclei that, when decaying, emit energy as radiation in the form of particles (e.g. α particles, an electron-electron antineutrino pair or a positron-electron neutrino) as well as electromagnetic radiation. Due to the very diverse penetration of the above forms of energy, and therefore the probability of interaction with matter, their effects can be strong, even if they are not in direct contact with organisms. Moreover, due to the possibility of creating stable compounds, radioisotopes are considered high-risk pollutants which, even in relatively low concentrations, can have a strong impact on organisms, posing a threat to the functioning of the environment at specimen, species and population level.

1.1 Sources of natural radioisotopes

Radioisotopes occurring naturally on Earth are the result of the interaction of cosmic radiation with atoms in the atmosphere, constituting the so-called cosmogenic radioisotopes (e.g. ^{14}C, ^{7}Be), but also main primordial radioisotopes with very long half - lives and members of the decay chains (e.g., ^{238}U, ^{237}Np, ^{235}U, ^{232}Th,) and their products included in the decay chains such as ^{222}Rn, ^{210}Po, ^{230}Th or ^{210}Pb (Gaffney & Marley, 2006). They are released during volcanic eruptions and as a result of rock weathering processes (Santhanabharathi et al., 2023). Their share has increased since mining of uranium ores, but also through the burning of fossil fuels and the extraction of fertilizers, mainly phosphate fertilizers, which ultimately leads to the concentration of natural radioisotopes in the environment through intensive fertilization (Landa, 2007). From the point of view of radiation protection, the most important are the daughter isotopes of the uranium-radium series, in particular ^{222}Rn. This only noble gas in the uranium-radium series enters the atmosphere from uranium deposits, where it can be inhaled by organisms, but also due to its short half-life ($t_{1/2} = 3.8$ days), it quickly transforms to ^{210}Pb, from other radioisotopes with a short half-life in between (Baskaran, 2011; Vogiannis & Nikolopoulos, 2015). Lead-210 ($t_{1/2} = 22.3$ years) effectively adsorbed to submicron aerosols minerals in the atmosphere which are known to be the most important transporters of this radioisotopes in the atmosphere (Isakar et al., 2015). After fallout from the atmosphere ^{210}Pb tends to have a strong affinity for soil or sediment and became a source of ionizing radiation (IR) in land ecosystems (Rastogi & Sarin, 2008).

1.2 Sources of anthropogenic radioisotopes

With the progress of research in the field of nuclear physics and as a result of strong political pressure related to World War II and then Cold War, since 1945 significant amounts of anthropogenic radionuclides were released into the environment and the levels of radioisotopes considered natural, such as ^{3}H and ^{14}C, increased (Gaffney & Marley, 2006; Hu et al., 2010; Mietelski, 2010). The increase in radioactivity resulting from human activity began to be significant on a local and global scale, posing a potential threat to organisms.

In total, about 2 000 nuclear weapons tests were conducted, including over 500 in the atmosphere (Mietelski, 2010). Radioisotopes released during atmospheric explosions were distributed throughout the planet, but most were deposited in the Northern Hemisphere due to the location of the test sites and the separation of air flows between the hemispheres (Hu et al., 2010; Kuśmierczyk-Michulec & Baré, 2024). However, radioisotopes could spread over long distances and then deposited as a global fallout radioisotopes. Due to high mobility, nuclear-related contaminants have not only become a threat at detonation sites, but also potentially in places considered untouched by human activity. Besides of military sources, significant amounts of radioisotopes have been released during accidents at nuclear power plants such as Chernobyl (former USSR, March 1986), Fukushima Daiichi (Japan, March 2011), and also to a lesser extent during failures with a lower level of threat, such as Three Mile Island (USA, March 1979). In addition, non-weapons test military accidents, such as nuclear transport disasters (Lind et al., 2007), but also space agency accidents involving satellites containing radioisotope thermoelectric generators, e.g., the breakup of SNAP-9A over the coast of East Africa (Rääf et al., 2017), resulted in the release of radionuclides into ecosystems. Despite strong control by international organizations and a significant reduction in the number of nuclear tests in connection with the Partial Nuclear Test Ban Treaty signed in 1963 by the largest emitters of anthropogenic radioisotopes, the legacy of these tests persists in the global ecosystem to this day, due to the relatively long half-life of some radioisotopes (Ikeda-Ohno et al., 2016; Werner & Purvis-Roberts, 2007).

Non-metallic anthropogenic and also natural radioisotopes released in gaseous form into the atmosphere, such as ^{3}H, ^{14}C or ^{131}I, spread to long distances from the sites of nuclear weapon explosions. An increase in ^{14}C activity concentrations has been observed around the world, but atmospheric concentrations have now fallen to near pre-1945 levels (Hodge et al., 2000). ^{131}I is an isotope of particular importance due to its ability to bind to the thyroid gland, however, due to its short half-life ($t_{1/2}$ = 8.02 days) its activity concentration in ecosystems is currently low (Tab. 1). From the point of view of current radiation protection, radioisotopes with relatively medium and long half-lives, such as ^{90}Sr, ^{137}Cs, $^{238-240}$Pu, or ^{241}Am, are important. These metallic radioisotopes, which are cations, released into the atmosphere as a result of nuclear military activity and nuclear power plant accidents, were associated with the mineral fraction in the air and then spread in the troposphere and stratosphere, falling in the form of global fallout.

Table 1. Total activity (PBq) and average cumulative fallout (Bq/m^{2}) for selected radioisotopes from atmospheric nuclear weapons tests.*value calculated to 2009 according to Mietelski (2010)

Isotope	**$t_{1/2}$**	**Total activity (PBq)**	**Average cumulated fallout* (Bq/m^{2})**
^{3}H	12.33 y	186 000	42 700
^{14}C	5 730 y	213	680
^{89}Sr	50.5 d	117 000	0
^{90}Sr	28.63 y	622	643
^{131}I	8.02 d	675 000	0
^{137}Cs	30.07 y	948	1035
^{239}Pu	24 110 y	6.52	21
^{240}Pu	6 563 y	4.35	14
^{241}Pu	14.35 y	142	47

1.3 Risk related to radioisotopes in the environment

The spread of radioisotopes depends on the source, ionic form and state of matter, and its mass, which ultimately affects their mobility in the environment and bioavailability, and therefore their potential impact on organisms (Baskaran, 2011; Kuśmierczyk-Michulec & Baré, 2024; Landa, 2007; Ohnishi, 2012; Rääf et al., 2017). When estimating the risk associated with radioisotopes in the environment, it is important not only to assess the concentration, but also to analyse the composition of radioisotopes. This strategy allow for the assessment of threats that vary depending on the radiation characteristics, the spatial location of a given isotope in relation to organisms and its chemical properties. Metallic radioisotopes bind effectively to mineral and organic compounds, but the strength of this bond varies greatly depending on environmental conditions and the elements themselves. Very heavy radioisotopes, such as Uranium or transuranium isotopes, are absorbed by organisms to a very low extent; in the case of these isotopes, almost 100% of the radioisotope relationship with organisms results from adsorption to their surfaces (Fisher et al., 1983; Panak & Nitsche, 2001). Considering the threats posed by radioisotopes in the context of chronic long-term radiation, those with lower atomic weight, higher activity concentration and relatively long half-life, such as ^{137}Cs or ^{210}Pb, seem to be particularly important.

Lead-210 ($t_{1/2}$ = 22.3 years) is a natural metallic radioisotope that emits mainly electrons through β^- decay, and as a result, the excited ^{210}Bi nucleus emits low-energy γ radiation with an energy of 46.6 KeV. Lead-210 is produced by the decay of ^{222}Rn leaking from Earth's uranium deposits and as a divalent metallic cation, has a strong affinity for minerals and organic matter. Lead can be transported to eukaryotic cells through divalent ion transporters and Ca^{+2} channels (Bannon et al., 2002; Kerper & Hinkle, 1997), but in the case of bacteria, its passage through the cell wall and membranes is limited (Chen et al., 2015; Tornabene & Edwards, 1972).

Cesium-137 ($t_{1/2}$ = 30.07) is an anthropogenic metallic radioisotope that emits mainly electrons by β^- decay, and as a result, the excited ^{137m}Ba nucleus emits γ radiation with an energy of 661.7 KeV. Its source is the fission processes of ^{235}U nuclei. Cesium-137, as a metallic cation, also has a strong affinity for minerals, forming stable complexes with them (Cornell, 1993; Park et al., 2021), however, due to its chemical similarity to K^+, it is effectively transported to both eukaryotic cells (Iwamoto & Shiraiwa, 2016; Topcuoğlu, 2001) and prokaryotes (Ivshina et al., 2002).

1.4 Interplay between ionizing radiation and organisms

Ionizing radiation can affect organisms directly and indirectly. Directly, α and β particles (mainly electrons) cause strong ionization of organic compounds in cells (Reisz et al., 2014; Sowa et al., 2006). As a result, structural or enzymatic proteins are dysfunctional and RNA and DNA are interrupted (Goodhead, 1994; Reisz et al., 2014; Sowa et al., 2006). The indirect path mainly involves the effects of radiolysis of water contained in cells or in the immediate surroundings. Due to the fact that water is the main component of all organisms, the probability of energy deposition through water radiolysis is high (Reisz et al., 2014). Radiolysis is a process in which IR ionize or excite water, resulting in the formation of particles with strong oxidative potential such as H_2O_2, HO• or reducing properties such as unpaired electrons or H• (Leach et al., 2001; Reisz et al., 2014). Indirect effects of IR on organisms also include dysfunction of cellular structures, mainly DNA transcription mechanisms, plastids. Mitochondrial dysfunction leads to increased secondary formation of reactive oxygen species and reactive nitrogen species, which subsequently cause structural and metabolic dysfunction of cells (Kam & Banati, 2013; Leach et al., 2001).

The effects of high doses of IR are well known in many groups of organisms (Caplin & Willey, 2018; Reisz et al., 2014; Sowa et al., 2006). However, events emitting such high doses are rare, while in many places on Earth, increased IR is observed, and organisms living there may experience low-dose chronic IR (Geras’kin et al., 2021; Nybakken et al., 2023). Currently, research in such conditions was carried out in areas with naturally increased radioactivity, and in places of former nuclear power plant accidents or nuclear weapons test sites. In the area of increased radioactivity near Chernobyl, a negative impact of chronic radiation on human health was observed, in particular an increased incidence of proliferative atypical cystitis (Romanenko et al., 2009), thyroid cancer and leukemia (Moysich et al., 2002). In the same area, IR affected lipid metabolism, the immune system, overall gut health and the gut microbiota of small rodents (Jernfors et al., 2024; Kesäniemi et al., 2019). Moreover, increased genetic diversity and increased levels of DNA damage were observed in scots pine (*Pinus sylvestris*) along the IR gradient near Chernobyl (Geras’kin & Volkova, 2014; Nybakken et al., 2023), but cytogenetic and phytohormonal changes were also observed in pine red (*Pinus resinosa*) in Fukushima Prefecture (Geras’kin et al., 2021). Despite this evidence, we lack information on how nuclear power plant accidents, but to a greater extent radioactivity resulting from nuclear weapons testing, may affect organisms outside test sites and areas closely related to nuclear power plant disasters. In recent decades, the glacial surface has been characterized as an ecosystem with a relatively high content of radioisotopes subjected to global fallout.

1.5 Elevated radioisotope concentrations on the surface of glaciers

Glaciers are considered to consist of diverse ecosystems (Hodson et al., 2008). The most biologically active and most strongly linked with the atmosphere is the supraglacial ecosystem (the surface of glaciers), dominated by microbial communities. Organisms on glacier surfaces are under strong pressure from abiotic factors, such as low temperature, relatively high UV radiation (mountain glaciers), and frequent freeze-thaw cycles. Air transport is one of the strongest factors influencing the supraglacial ecosystem, supporting the colonization of glaciers, delivering organic matter and minerals, but also pollutants such as pesticides, microplastics or heavy metals and radionuclides (Ambrosini et al., 2019; Pautler et al., 2013; Pittino, Buda, et al., 2023; Xu et al., 2010).

Biomass and pollutants on the surface of glaciers concentrate in a dark sediment called cryoconite. The term was introduced by Finnish explorer Adolf Nordenskiöld and origin from Greek words, κρύος – cold and κόνις – dust. This sediment is formed from a mixture of minerals and organic matter, mainly as a result of the activity of glacial cyanobacteria (Fig. 1), which produce extracellular polymeric substances causing the binding of mineral and organic fractions (Takeuchi et al., 2010; Wejnerowski et al., 2023). Cryoconite melts into the ice, creating water-filled depressions called cryoconite holes (Fig. 1), and can occur in a dispersed form directly on the ice surface or in the border between the ice and rock debris on the glacier surface (Fig. 1). This dark sediment is biological hotspots on the surface of glaciers worldwide, while dark colour is related to the organic pigments. Rozwalak et al., (2022) have shown that the darkness of cryoconite is strongly linked to the organic matter content, which varies from about 1% to over 30%. In turn, this has strong implications for glaciers in general because the cryoconite is responsible for the efficient absorption of solar radiation and, as a result, the melting of glaciers, but also provides liquid water for organisms (Di Mauro et al., 2017; Li et al., 2019).

Cryoconite is dominated by heterotrophic and autotrophic bacteria, fungi, algae and heterotrophic protozoa (Buda, Łokas, et al., 2020; Cameron et al., 2012; McCrimmon et al., 2018; Pittino, Zawierucha, et al., 2023). The composition of microbial communities changes between years and on an annual scale, at the beginning of the summer season, the surface of glaciers is a highly photoautotrophic ecosystem, then turns into a heterotrophic system at the end of summer (Pittino et al., 2018). The surface of glaciers is generally nutrient-poor, however, organisms inhabiting cryoconite holes are adapted to use diverse carbon sources dependent on temporal variation (Pittino, Zawierucha, et al., 2023; Poniecka et al., 2020); this again underlines an essential role of atmospheric delivered matter in the functioning of the supraglacial ecosystem. Temporal niche variation that stimulates overall biodiversity is also influenced by the formation of anaerobic zones of cryoconite, both in the vertical profile of the sediment and on a micro-scale of cryoconite granules, where the inner parts are anaerobic (Buda et al., 2022; Poniecka et al., 2018; Segawa et al., 2020). Top consumers do not follow this microbial diversity. Over the globe, cryoconite is dominated by two phyla of animals (Tardigrada and Rotifera), with local exceptions (Zawierucha et al., 2015). Their ecological significance is unclear so far. Nevertheless, considering their relatively high densities, where they can reach over 1000 individuals per one ml of wet sediment (personal observations), their ecological significance seems to be crucial. Tardigrades dominate cryoconite on glaciers in the Alps, while springtails dominate the surface. The latter can reach over ten million individuals on the tongue of a small Alpine glacier (Buda, Azzoni, et al., 2020). Glacier surface biota have to live in extreme conditions such as permanent low temperatures and frequent thaw-freeze cycles. However, it was pointed out that now they are subject to another pressure related to atmospheric-delivered pollutants concentrated by cryoconite.

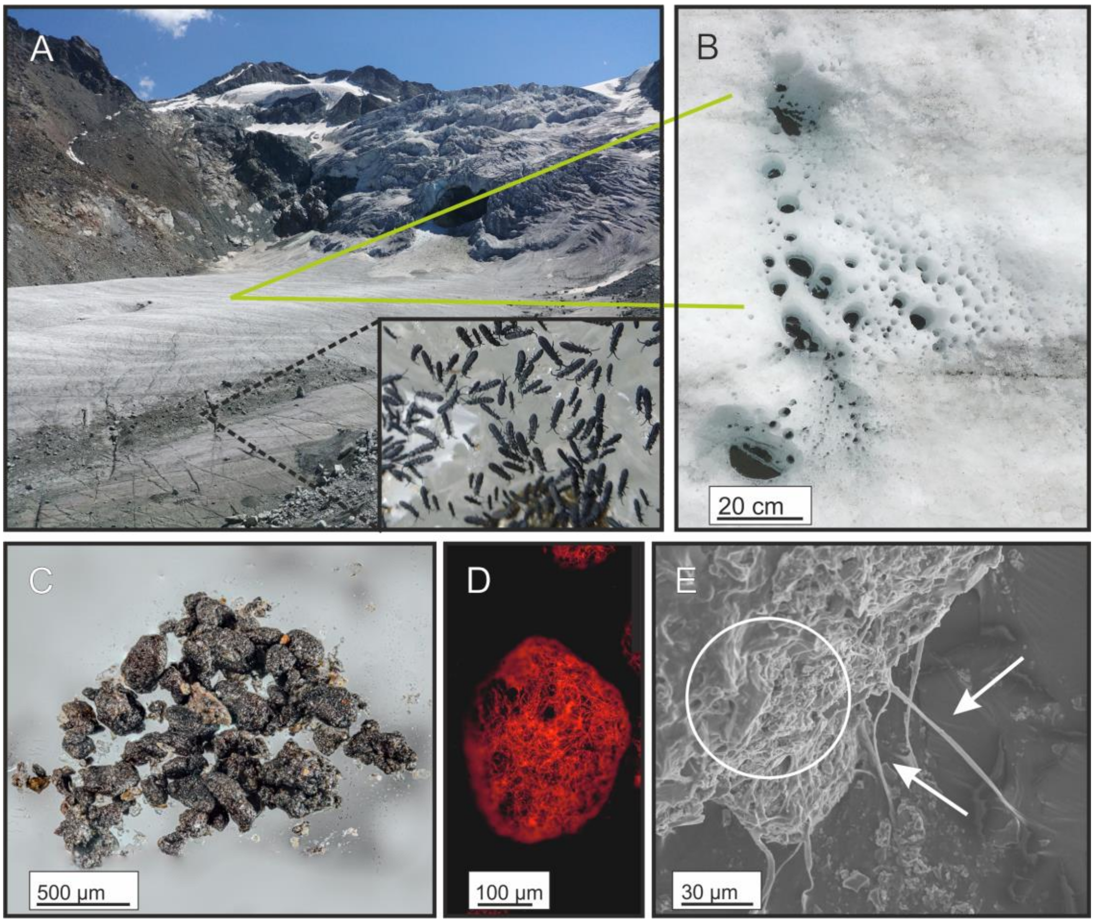

Figure 1. Supraglacial ecosystem. Ablation zone of an alpine glacier (A) with marked area of cryoconite holes (B) and debris cover with springtails under stones (inset). Dark cryoconite sediment (C) from the bottom of cryoconite holes, single cryoconite granule under a fluorescence microscope (blue light excitation) with visible cyanobacteria (D). Experimentally formed cryoconite granules with visible cyanobacterial filaments (arrows) and biofilm (circle), photo from a scanning electron microscope. Photo C thanks to courtesy of Szymon Kawecki. Photo E from the Wejnerowski et al. 2023.

Relatively high concentrations of natural (^{210}Pb) and anthropogenic (^{137}Cs, $^{239+240}$Pu; Fig. 2) radionuclides were found on glaciers located in mountains and polar regions (Baccolo et al., 2020; Buda, Łokas, et al., 2020; Clason et al., 2023; Łokas et al., 2018). The main source of anthropogenic radioisotopes on glaciers is global fallout resulting from atmospheric nuclear weapons tests, but also local sources such as the Chernobyl nuclear power plant disaster, the breakup of the SNAP-9A generator from satellite, and local atmospheric nuclear weapons tests, as in the case of radioisotopes on glaciers in Caucasus (Baccolo et al., 2020; Buda, Łokas, et al., 2020; Łokas et al., 2018, 2022). Fallout radioisotopes on the surface of glaciers are effectively captured by cryoconite spread in the ablation zone (Łokas et al., 2022; Owens et al., 2023) reaching average activity concentrations from 11 to 37 388 Bq kg^{-1} for ^{137}Cs and from 247 to 11 764 Bq kg^{-1} for ^{210}Pb in the northern hemisphere and from 3 to 315 Bq kg^{-1} for ^{137}Cs and from 7 to 1 853 Bq kg^{-1} for ^{210}Pb in the southern hemisphere (Clason et al., 2023). The activity concentrations of natural (^{210}Pb) and anthropogenic (^{137}Cs, $^{238-240}$Pu, ^{241}Am) radioisotopes in cryoconite are much higher than in the surrounding habitats (Baccolo et al., 2020; Clason et al., 2021; Łokas, Wachniew, et al., 2017; Owens et al., 2019).

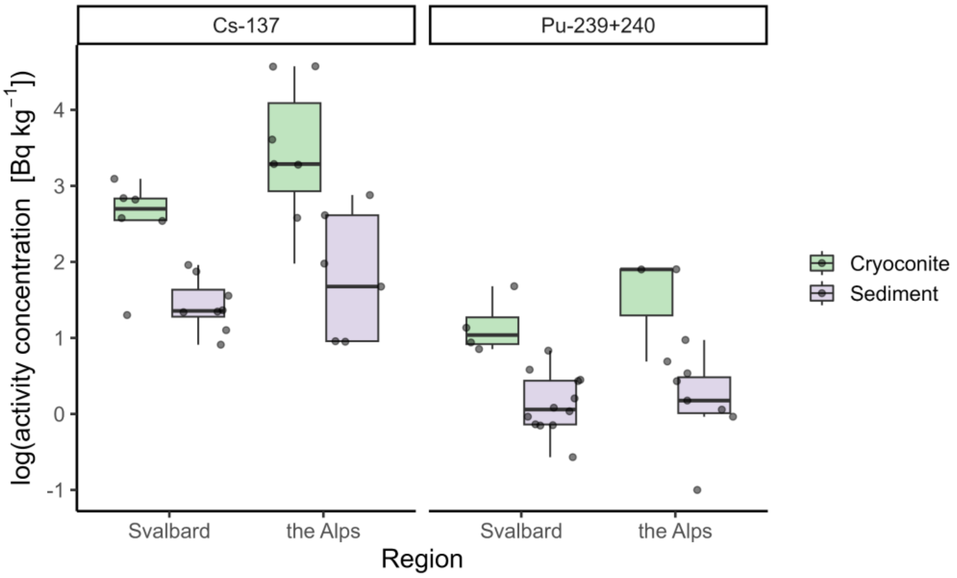

Figure 2. Comparison of anthropogenic radioactivity in cryoconite and in non-glacial sediments (lake bottom sediments, peat bogs, soil) on Svalbard and the Alps. ^{137}Cs activity concentrations have been calculated for 2020. Data obtained from Łokas et al. (in prep), Alewell et al. (2014); Breban et al. (2003); Ciuffo et al. (2002); Clason et al. (2023); Corcho-Alvarado et al. (2016); Leclercq et al. (2015); Łokas, Anczkiewicz, et al. (2017); Łokas et al. (2013); Łokas, Zwoliński, et al. (2017); Tieber et al. (2009).

1.6 Thesis objectives

Knowing that the surface of glaciers is under high pressure of elevated radioactivity due to the accumulation of fallout radionuclides in biogeochemical hotspots – cryoconite holes – the main goal of this thesis is to understand whether elevated concentrations of anthropogenic and natural radioisotopes may pose a threat to organisms inhabiting glaciers and surrounding ecosystems.

Thesis is form of three integrated parts:

The first chapter focuses on processes related to accumulating fallout radioisotopes in cryoconite on a glacial scale. In particular, activity concentrations of artificial (^{137}Cs) and natural (^{210}Pb) radioisotope variation are analysed along with variables that can affect their accumulation based on cryoconite ecological studies. These are the organic matter content in the cryoconite, chlorophyll concentration, the relative abundance of Cyanobacteria, and the size of aggregates and minerals. Moreover, because in the case of radioactivity, important spatial relation with organisms, the mobility of ^{137}Cs and ^{210}Pb was assessed by measuring their activity concentrations after a parallel extraction using media with different specific ion exchange capacities presenting a bioavailable profile of these radionuclides. In the end, to understand whether organisms can accumulate radioisotopes, the uptake ratio of ^{137}Cs, ^{210}Pb, ^{238}Pu, and $^{239+240}$Pu in the top consumers was measured in springtails that inhabit the supraglacial zone of Alpine glaciers.

The primary goal of the second chapter was to assess the risk associated with environmental radioactivity on a regional scale by analysing the factors influencing the accumulation of radioisotopes (^{137}Cs, ^{210}Pb and ^{241}Am) and analysing the possible fate of the accumulated radioisotopes during the expected intense melting of glaciers in the Alps. It was achieved by measuring activity concentrations on 19 glaciers across the Alps and analysing their spatial variation. Glacier features include average altitude, surface area, and organic matter content in the cryoconite. Moreover, given that the accumulation of ^{137}Cs and ^{241}Am from global atmospheric fallout could potentially be influenced by precipitation, past precipitation on the glaciers was included in the analysis. This approach, combined with spatial autocorrelation analysis, allows us to determine whether the observed high radioactivity on some glaciers is due to delivered amounts or related to glacier features that promote the concentration of pollutants on their surfaces. Overall, it led to understanding the fate of radioisotopes on different mountain glaciers and where we should expect radioisotopes hotspots after glaciers melt.

The third chapter analyses the relationship between activity concentrations of ^{137}Cs and ^{210}Pb and the biodiversity of microbial communities in cryoconite holes on alpine glaciers. The hypothesis was that due to the negative effects of IR, the biodiversity of organisms inhabiting cryoconite will decrease activity concentration of ^{137}Cs and ^{210}Pb increases. Analysis of bacterial and eukaryotic diversity was based on DNA amplicon sequencing of samples collected on 16 glaciers in the same year, which led to the exclusion of temporal variation of microbial compositions as it was reported that communities on glaciers can vary over seasons. Moreover, the cryoconite holes on the glaciers in the Alps are dominated by one Tardigrada genus, the only top consumer in alpine cryoconite holes. This allowed to assess whether the effects of microbial diversity in lower-trophic groups can be visible by the change in the population abundance of top consumers in cryoconite.

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CHAPTER I

Unveiling threats to glacier biota: bioaccumulation, mobility, and interactions of radioisotopes with key biological components

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CHAPTER II

Small glaciers as radioactive hotspots: concentration of radioisotopes during predicted intensive melting in the Alps

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(under review)

Abstract

The primary goal of this chapter was to explain what factors can affect the accumulation of fallout radionuclides in cryoconite at regional scale (the Alps). The focus was on the relationship between altitude, surface area of glaciers, organic matter content in cryoconite, precipitation, and the activity concentrations of natural (^{210}Pb) and anthropogenic radionuclides (^{137}Cs and ^{241}Am). The activity concentrations on 19 sampled glaciers were comparable to previous findings in the Alps but showed large variability. ^{137}Cs and ^{210}Pb activity concentration increased with the organic matter content (^{137}Cs: $F = 6.4$, $df = 1$, $p = 0.022$; ^{210}Pb: $F = 69.962$, $df = 1$, $p < 0.001$), emphasizing the importance of biotic-abiotic interactions in pollutant accumulation within the cryoconite ecosystem. The activity concentration of ^{210}Pb decreased with glacier altitude ($F = 1.495$, $df = 1$, $p = 0.039$), possibly reflecting atmospheric variations in ^{222}Rn, the indirect parent isotope of ^{210}Pb. The results indicate that precipitation events, such as during a peak in ^{137}Cs deposition in the 1960s or a month after the Chernobyl nuclear power plant accident (1986), did not directly impact current activity concentrations. The lack of spatial autocorrelation of radioisotope activity concentrations between glaciers further supports the results. Importantly, this study shows that the activity concentrations of the investigated radioisotopes in cryoconite were higher on smaller glaciers (^{210}Pb: $F = 5.21$, $df = 1$, $p = 0.037$; ^{137}Cs: $F = 4.878$, $df = 1$, $p = 0.042$; ^{241}Am: $F = 5.208$, $df = 1$, $p = 0.039$). This directly supports the hypothesis that the cryoconite retains a significant share of radioisotopes stored in the ice during intensive melting. Since many small alpine glaciers are predicted to disappear within the next 50 years, the release of radioisotopes to mountain ecosystems might be higher than previously forecasted, creating a risk for glacier and glacier-adjacent ecosystems.

Small glaciers as radioactive hotspots: concentration of radioisotopes during predicted intensive melting in the Alps

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Introduction

The record of intensive anthropic pressure, such as the legacy of industry and military activities, is present in diverse habitats across Europe (Gabrieli et al., 2011; Gallini et al., 2018; Panagos et al., 2013). Among the high-risk pollutants, anthropogenic radioisotopes persist in different habitats at relatively high activity concentrations, even if the amount released has dropped significantly since the Limited Test Ban Treaty in 1963 (Baccolo, Łokas, et al., 2020; Foucher et al., 2021; Gabrieli et al., 2011; Krouglov et al., 1998; Łokas et al., 2022; Mietelski, Maksimova, et al., 2010). The main sources of anthropogenic radioisotopes in Europe are local and global nuclear weapon tests (known as global fallout) and, to a large extent, the Chernobyl Nuclear Power Plant (NPP) accident (Gabrieli et al., 2011; Łokas et al., 2018). Some radionuclides are harmful when ingested or inhaled in high amounts but pose low threats in terms of long-term effects on the environment overall. Two of them, ^{131}I and ^{89}Sr, are especially important for human health, but because of their very short half-life ($t_{½}$ = 8.04 and 50.52 days, respectively), the removal rate from the environment due to natural decay is fast. Therefore, the long-term effects are limited (Minenko et al., 2022). On the other hand, some radioisotopes persist in the environment and accumulate in higher trophic organisms due to their longer half-life and chemical properties (Copplestone et al., 1999; Dragović & Mandić, 2010; Mietelski, Dubchak, et al., 2010). Therefore, the most important in terms of biological threats are radioisotopes released in high amounts but also with relatively long half-lives such as ^{90}Sr ($t_{½}$ = 28.78 yrs.), ^{137}Cs ($t_{½}$ = 30.17 yrs.) or ^{239}Pu ($t_{½}$ = 24 110 yrs.) along with other Plutonium-related isotopes, such as ^{238,240,241}Pu (^{238}Pu: $t_{½}$ = 87.7 yrs., ^{240}Pu: $t_{½}$ = 6561 yrs., ^{241}Pu: $t_{½}$ = 14.3 yrs.) or ^{241}Am ($t_{½}$ = 432 yrs.). Cesium-137 was released by NPP accidents and global military actions, with a peak in deposition in 1963 (Hu et al., 2010). Since ^{137}Cs reach high activity concentrations, their chemical properties are similar to those of potassium, and their decay produces beta particles, there is a high concern about its presence in the environment (Ashraf et al., 2014; Singh et al., 2022). Its primary sources have significantly dropped after the Limited Test Ban Treaty, and its activity concentrations are progressively decreasing, with some peaks in activity due to NPP accidents (Foucher et al., 2021). However, other longer-lived anthropogenic radioisotopes, such as ^{241}Am or $^{238\text{-}240}$Pu, persist in amounts similar to those observed when released (Liao et al., 2014). Their radioactivity's effects on ecosystems are still observed but only well investigated in areas close to NPP accidents, where elevated ionising radiation exerted broad effects on wildlife. For instance, close to the Fukushima Daichi NPP accident, increased aberrant cell frequencies were found in pine needles exposed to higher IR doses (Geras'kin et al., 2021) and decreases in bird abundances were observed due to chronic exposure (Garnier-Laplace et al., 2015). Furthermore, scots pines *Pinus sylvestris* exposed to elevated levels of ionising radiation in the vicinity of the Chernobyl NPP accident exhibit increased DNA damage (Nybakken et al., 2023). Additionally, bank voles *Clethrionomys glareolus* have shown a response to this radiation by reducing overall gut health (Jernfors et al., 2024) along with genomic scale alterations (Jernfors et al., 2021).

Due to their position and relatively intense precipitation, the Alps have received high amounts of anthropogenic radionuclides from global fallout and the Chernobyl NPP accident (Erlinger et al., 2008; Gerdol et al., 1994). Relatively high activity concentrations in the Alps were found on the surface of glaciers (Baccolo et al., 2017; Baccolo, Nastasi, et al., 2020; Tieber et al., 2009). The dark sediment called cryoconite, which covers glaciers worldwide is a biogeochemical hotspot that adsorbs contaminants delivered with dry and wet deposition from the atmosphere (Baccolo et al., 2017; Buda et al., 2024; Clason et al., 2023; Łokas et al., 2022; Pittino et al., 2023). Cryoconite is composed of minerals, microbial communities, and a high share of dead organic matter that originates on the glacier (autochthonous organic matter) or elsewhere (allochthonous organic matter) (Pautler et al., 2013; Pittino et al., 2018; Rozwalak et al., 2022; Takeuchi et al., 2010). Due to its dark colour, the sediment absorbs solar radiation and melts into the ice, creating water-filled depressions called cryoconite holes (Cook et al., 2016; McIntyre, 2011; Rozwalak et al., 2022). This habitat is dominated by microorganisms such as bacteria, algae, fungi and other eukaryotic groups, including heterotrophic protozoa or microinvertebrates, no vascular plants or vertebrates live and reproduce there (Buda et al., 2020; McCrimmon et al., 2018; Pittino et al., 2018; Zawierucha et al., 2022). Organisms producing extracellular polymeric substances like Cyanobacteria, which are highly abundant in cryoconite, seem to play an important role in binding the mentioned cryoconite compounds, but potentially also the contaminants into spherical granules (Rozwalak et al., 2022; Takeuchi et al., 2010; Wejnerowski et al., 2023). These bioaggregates were found to be important in the analysis of sources of radioactivity globally, even in remote areas, due to the capability of cryoconite to accumulate radionuclides which also provides a new substratum for environmental monitoring

(Baccolo, Nastasi, et al., 2020; Buda et al., 2020; Clason et al., 2023; Łokas et al., 2016; Owens et al., 2023; Tieber et al., 2009; Wilflinger et al., 2018).

On Alpine glaciers, the main sources of anthropogenic radionuclides were global fallout (atmospheric nuclear blasts) with a peak in the '60s and the Chernobyl NPP accident in 1986 (Baccolo, Łokas, et al., 2020; Tieber et al., 2009; Wilflinger et al., 2018). The concentration of anthropogenic isotopes estimated for 1986 reached 223 kBq kg^{-1} of ^{137}Cs and 129.1 kBq kg^{-1} of ^{134}Cs (Wilflinger et al., 2018). On the other hand, the activity concentration of the most abundant natural isotope found in cryoconite – ^{210}Pb – was equal to 57 kBq kg^{-1} when estimated for 1986 (Wilflinger et al., 2018). The overall comparison of mean activity concentrations between cryoconite and other Alpine habitats in the Alps has shown that both natural and anthropogenic radioisotopes are higher in the former by one to two orders of magnitude than in surrounding ecosystems (Baccolo, Łokas, et al., 2020). These works are important for understanding pollution levels and radionuclide sources in the Alps but were limited to a few glaciers. The lack of large-scale data lowers our ability to infer spatial variation and factors that affected the accumulation in the Alps. Indeed, the high variation in activity concentrations observed between the investigated glaciers raises a question of whether the accumulation is mainly driven by spatial variation (e.g. distance from the source or variation in precipitation) or glacier-related features. Recognition of these processes might be important in estimating the effects of ongoing intense glacier melting for the release of radioactive contaminants accumulated over time, some of which, like ^{239}Pu or ^{241}Am, persist in the environment over an extended time.

In this research, we examine the accumulation and concentration of radioisotopes on glaciers at a regional scale. Our analysis is based on cryoconite sediment samples collected from 19 glaciers in the Alps over two years. We specifically focused on two anthropogenic radioisotopes (^{137}Cs and ^{241}Am) and one natural radioisotope (^{210}Pb). These radioisotopes are the most radioactive fallout isotopes on glaciers, widely detectable by gamma spectrometry, and known to concentrate well in cryoconite compared to other habitats (Baccolo et al., 2019; Clason et al., 2023; Łokas et al., 2016). Therefore, they are the most important isotopes for analysing the threats related to the biota under intensive melting and the potential release of stored radioisotopes. We measured radioisotopes' spatial variation in activity concentrations and examined their relationship with glacier features such as average altitude, surface area, and organic matter content in the cryoconite. Moreover, given that the concentration of ^{137}Cs and ^{241}Am from the global atmospheric fallout could be influenced by precipitation, we also analysed their activity concentrations in relation to past precipitation on the glaciers. This approach, combined with spatial autocorrelation analysis, allows us to determine whether the observed high radioactivity on some glaciers is due to past precipitations or related to glacier features that promote the concentration of pollutants on their surfaces. This comprehensive study focused on a single geographical area (the Alps). It thus controlled for unknown parameters such as large variations in ^{222}Rn sources (an indirect parental isotope of ^{210}Pb), large-scale air masses flow, and the composition of minerals and organisms, thereby enhancing the power of our inferences. This could be beneficial for future predictive analyses of ongoing global changes and the potential release of secondary pollutant sources due to glacier melting.

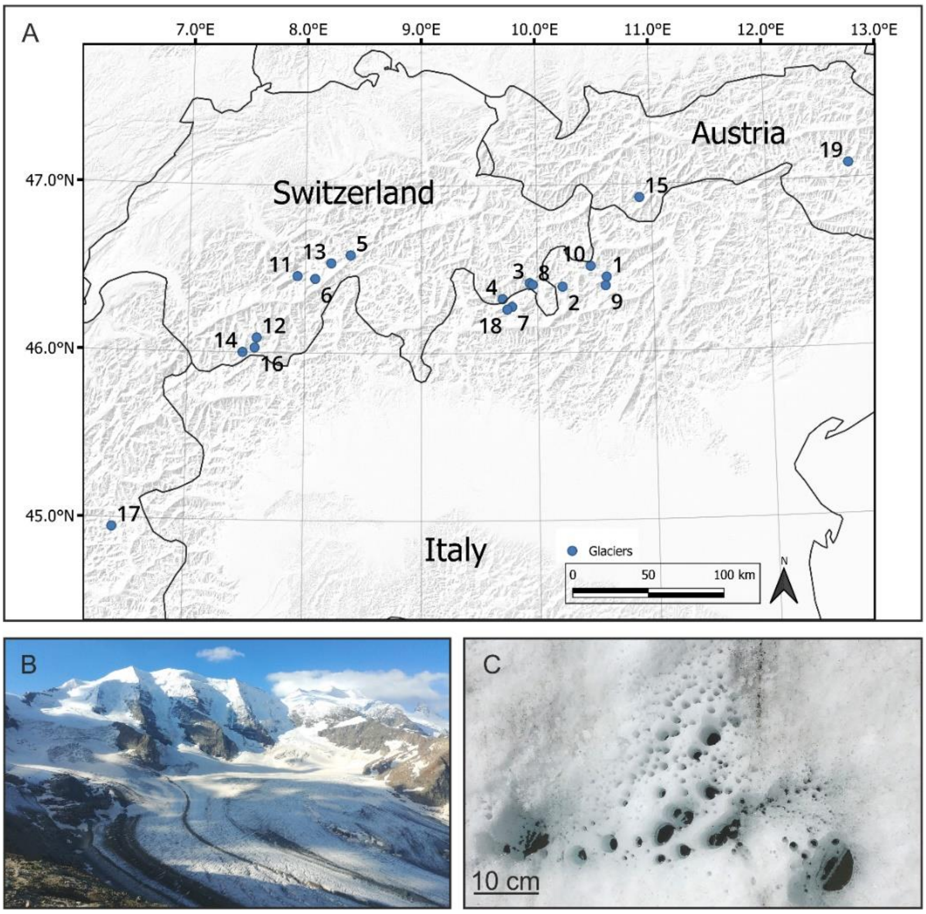


Figure 1. Map of the Alps with sampled glaciers (blue points). Pers Glacier (B), cryoconite holes (C) on de Moiry Glacier surface. The ID corresponds to the numeration in Supplementary Table 1 and 3.

Methods

Study area and sampling

In this study, our focus was on the most glaciated mountain groups in the Alps. We collected a total of 140 samples from 19 different glaciers during the 2020 and 2021 campaigns (Fig. 1). The altitude of the glacier sampling zones (ablation areas) ranged from 2015 to 3004 m a.s.l with a mean elevation of 2569 m a.s.l. A list of the investigated glaciers and their features is provided in Supplementary Table 1. We collected five to ten cryoconite samples from distinct cryoconite holes using a sterile clean pipette or a stainless-steel spoon from each glacier. We ensured that the cryoconite holes sampled were not interconnected. The collected samples were frozen at -20°C or preserved with ethyl alcohol. Upon returning to the laboratory, the samples were homogenised and dried in preparation for radiometric analysis. The average dry mass of the cryoconite samples used in the analysis varied from 0.462 g to 2.134 g, with a mean value of 0.948 g.

Radiometric analysis

Using gamma spectrometry, we estimated the activity concentrations of three fallout radioisotopes, namely ^{137}Cs, ^{210}Pb and ^{241}Am. These radioisotopes are known to be effectively concentrated in cryoconite compared to lithogenic radioisotopes such as ^{40}K, ^{238}U, ^{232}Th, ^{234}Th or ^{212}Bi (Baccolo, Łokas, et al., 2020). These radionuclides are related to their contents in the source minerals (Megumi et al., 1988) and are not influenced by the action of cryoconite. Lead-210 is also a lithogenic radioisotope as a product of the ^{238}U series. However, its presence in the environment might be from two different distribution pathways. The first one is the weathering processes of minerals containing ^{210}Pb (supported ^{210}Pb); this fraction is relatively low mobile and shows equilibrium with ^{238}U concentration in local minerals. The second fraction has atmospheric distribution, as the indirect parental isotope of ^{210}Pb, which is ^{222}Rn, a noble gas with a short half-life reaching the atmosphere from uranium-rich ores; this fraction is known as unsupported ^{210}Pb and can be transported in the atmosphere and then effectively captured. The vast majority of ^{210}Pb in cryoconite is concentrated from atmospheric deposition as unsupp. ^{210}Pb (Baccolo, Łokas, et al., 2020; Davidson et al., 2023), therefore, in our analysis, we relayed on total ^{210}Pb activity concentrations assuming that they represented mostly unsupp. ^{210}Pb. An example spectrum is presented in Supplementary Figure 1.

The samples were analysed with a low-background, digital gamma-ray spectrometer equipped with a Broad Energy Germanium (BEGe) detector BE5030 with a relative efficiency of about 48% and a multilayer passive shield surrounded by an active shield's detector (Gorzkiewicz et al., 2019). For ^{137}Cs, activity was determined by measuring the 661.6 keV emission peak of ^{137m}Ba, for ^{210}Pb, it was the 46.5 keV peak, while for ^{241}Am, the 59.5 keV peak was used as an analytical signal. Efficiency calibration (including self-absorption correction) for used measurement geometry was determined using LabSOCS calibration software (Mirion Technologies). The spectra were collected for approximately 24 h. The activity concentrations were then calculated on the day of sampling. Data quality was evaluated by measuring of IAEA Reference Materials (IAEA 447). The results (Supplementary Table 2) agreed well with the recommended values.

Predictors

We quantified organic matter as the percentage weight loss after combustion at 550°C for 6 h, following homogenisation and drying at 100°C for 24 h (Hoogsteen et al., 2018). The mass of the analysed samples was the same as that used for gamma spectroscopy, as the samples were combusted post-radiometric analysis.

We estimated the precipitation related to the peak of global fallout as the total precipitation in the glacier region from January 1962 to December 1964. Additionally, we estimated the precipitation that could influence the deposition of ^{137}Cs following the Chernobyl NPP accident as the total precipitation in May 1986. The data was gathered from WorldClim v2.1 (Fick & Hijmans, 2017; Harris et al., 2020) and proceeded in qGIS software (QGIS, 2023). We sampled the total precipitation for each month based on raster data from WorldClim, with sampled values for the coordinates of the sampling area. The surface area of glaciers, their average and maximum altitude was estimated for 2015/16, with one extent due to lack of data for 2015/16 (Pers, 2022), based on the Global Land Ice Measurements from Space (GLIMS) database (Raup et al., 2007). Detailed parameters of the glaciers and the results of the activity concentrations are presented in Supplementary Tables 1 and 3.

Statistical analysis

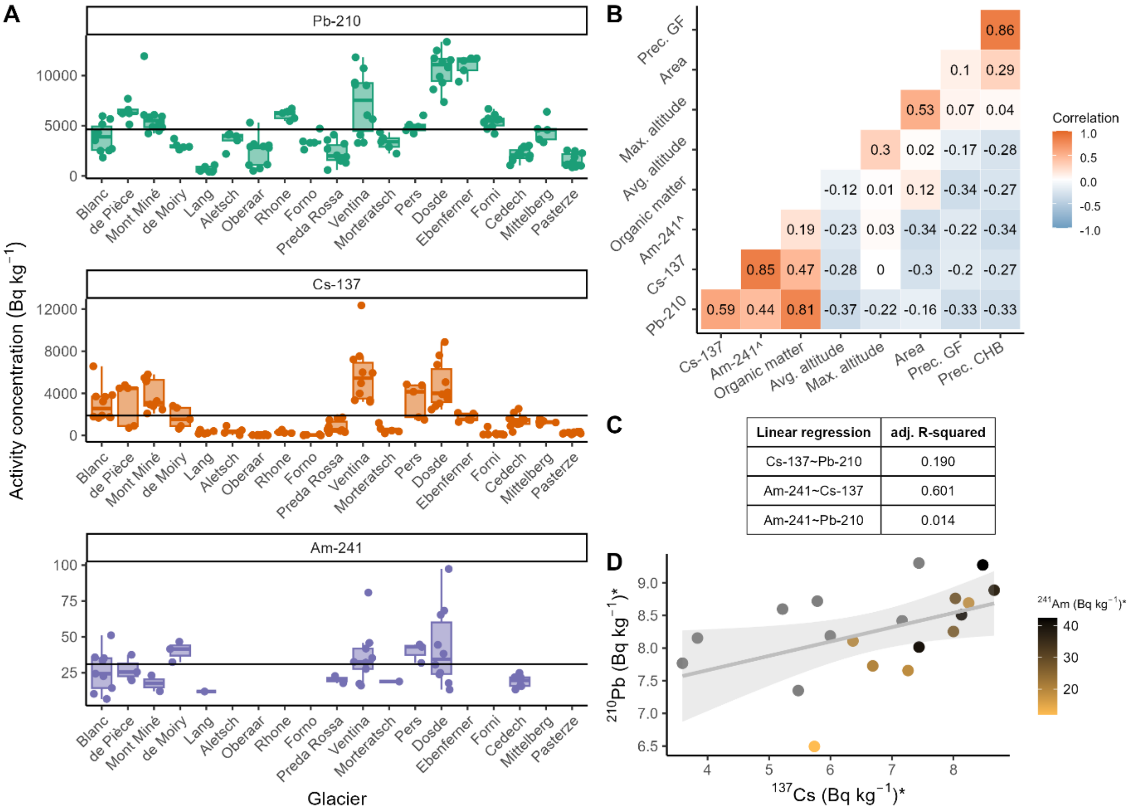
Radiometric data were averaged for each glacier, as our primary goal was to analyse factors that affect activity concentrations of radionuclides on an Alpine scale. First, we tested whether activity concentrations of radioisotopes are spatially autocorrelated between glaciers, which could be a proxy for weather-dependent deposition. This test was based on the k nearest neighbours estimate (k = 3, equal weights for each k) and the orthodromic distance between points, which are coordinates of sampling areas (one for each glacier). This spatial analysis was performed using the *sp* (Pebesma & Bivand, 2024) and *spdep* (Bivand, 2024) packages.

To determine which factors affect the activity concentrations of ^{210}Pb and ^{137}Cs, we used linear models with a Gaussian error distribution and log-scaled response variables. . Based on previous studies, we assumed that organic matter content (OM) is the most important predictor for 210Pb and 137Cs activity concentrations. Therefore, we built a set of models with OM as a core predictor and additional variables specific to each isotope. In the case of ^{210}Pb models, we built models that contained OM as the only predictor, OM with an additive effect of a) the surface area of the glacier, b) the average altitude of the glacier, and c) both the surface area and the average altitude of the glacier. For ^{137}Cs, we built models that contained OM as the only predictor, OM with an additive effect of a) the surface area of the glacier, b) the sum of precipitation during the peak of global fallout (GF), c) the sum of precipitation a month after the Chernobyl NPP accident, d) all the above predictors. The above models were compared separately for each radionuclide using an F test based on a reduction of the Residual Sum of Squares (RSS). The best model was chosen based on RSS reduction and presence-absence of influential observations. A comparison of models is presented in Supplementary Table 4, while only the results of the best models are presented in the results, with the test for predictor significance based on the F-test using Type-II Anova. In the case of ^{241}Am activity concentrations, on 8 out of 19 glaciers, we could not reach the minimum detectable concentration (MDC). Therefore, we used Censored Regression to analyse these data (Henningsen, 2022). This model contained the OM and the sum of precipitation during the peak of global fallout (GF) as predictors.

In the above models, the surface area of the glaciers and precipitation data were log-scaled to reduce the effect of influential observations; the Aletsch Glacier is extreme in size (~83.4 km^2) compared to the second largest glacier, Pasterze (16.2 km^2), while the precipitation on the Pasterze Glacier was four-fold higher than those on the second in order (see Supplementary Table 1).

Models were implemented in the R software v4.3.2 (R Core Team, 2024) using the *lm* function and the *censReg* (Henningsen, 2022) package. Linear models were checked for violation of assumptions based on diagnostic plots, and outliers' presence tested based on Cook's distances using *performance* package (Lüdecke et al., 2021). A project with raw data and R code is presented in the GitHub repository under the link: https://github.com/jakbud1/Chapter-II.git.

Results and Discussion



	Cs-137	Am-241^	Organic matter	Avg. altitude	Max. altitude	Area	Prec. GF	Prec. CHB
Prec. GF								0.86
Area							0.1	0.29
Max. altitude						0.53	0.07	0.04
Avg. altitude					0.3	0.02	-0.17	-0.28
Organic matter				-0.12	0.01	0.12	-0.34	-0.27
Am-241^			0.19	-0.23	0.03	-0.34	-0.22	-0.34
Cs-137		0.85	0.47	-0.28	0	-0.3	-0.2	-0.27
Pb-210	0.59	0.44	0.81	-0.37	-0.22	-0.16	-0.33	-0.33

Linear regression	adj. R-squared
Cs-137~Pb-210	0.190
Am-241~Cs-137	0.601
Am-241~Pb-210	0.014

Figure 2. Inter- and intra-glacier variation of ^{210}Pb, ^{137}Cs, and ^{241}Am activity concentrations across the Alpine glaciers ordered from West to East, solid lines on plots represent overall means (A). Correlation of variables included in the analysis, ^Values of ^{241}Am that were below MDC assigned as ½ MDC (B). Adjusted R^2 of linear relations between each pair of radioisotopes (C). Linear relation of ^{210}Pb on ^{137}Cs with ^{241}Am activity concentrations dependent on colour as the third dimension, grey dots are observations below MDC; *log-scaled vectors (D). Correlations between precipitations after GF and CHB NPP accidents are meaningful only for ^{137}Cs and ^{241}Am activity concentrations.

Radioisotopes pollution levels

The activity concentrations of ^{210}Pb, ^{137}Cs, and ^{241}Am in cryoconite were within the range of those previously reported on glaciers in the Alps (Baccolo, Nastasi, et al., 2020; Wilflinger et al., 2018), with an overall mean of 4 655 Bq kg^{-1}, 1 732 Bq kg^{-1} and 15 Bq kg^{-1}, respectively (Fig. 2). However, these activity concentrations are higher than those on glaciers in South America or Antarctica, but within the range of those found in the Northern Hemisphere (Clason et al., 2023). The lowest average activity concentration of ^{210}Pb was found on the Lang Glacier (661 Bq kg^{-1}), while for ^{137}Cs, it was on the Oberaar

Glacier (36 Bq kg^{-1}). The activity concentration of ^{241}Am was not detectable in any sample for 8 of the 19 glaciers, with a minimum average of 11 Bq kg^{-1} for glaciers that were above detection limits (which varied between 2.2 to 26 Bq kg^{-1}). In contrast, the highest activity concentration of ^{210}Pb was found on the Ebenferner (10 968 Bq kg^{-1}), ^{137}Cs on the Ventina Glacier (5 785 Bq kg^{-1}), and ^{241}Am on the Dosdé Glacier (40 Bq kg^{-1}). Average data are presented in Figure 2A and Supplementary Table 3. The high variation in activity concentrations between glaciers, even those closely located, without a clear spatial pattern, suggests that glacier-related processes are involved in accumulating natural and anthropogenic radioactivity on glaciers. While the correlations between the activity concentrations of each pair of radionuclides are positive (Fig. 2B), there is still a high proportion of unexplained variation, indicating that different mechanisms are involved in their accumulation on glaciers, possibly related to both sources and glacier features.

Effects of glacier-related features on activity concentration of radionuclides

Contrary to our expectation, neither the sum of precipitation during the GF peak nor the month following the Chernobyl NPP accident explained the current activity concentrations of ^{137}Cs (the best model based on the reduction of RSS did not contain these predictors) and ^{241}Am (F = 1.495, df = 1, p = 0.242; Tab. 1). The total precipitation varied, from 1840 mm to 5063.3 mm for the 3-years GF peak, and from 68.9 mm to 154 mm one month after Chernobyl NPP accident. This contradicts our hypothesis of an increase in the activity concentrations of ^{137}Cs with the sum of precipitation during the GF peak and in May 1986. The activity concentrations are calculated per 2020/21. However, this should have only implied lower values that vary linearly with precipitations. Indeed, a significant amount of ^{137}Cs released during the Chernobyl NPP accident was subject to wet deposition, strongly correlated with precipitation (Clark & Smith, 1988). Although precipitation was found to be the strongest predictor of ^{137}Cs deposition from global fallout (Pálsson et al., 2006), the lack of precipitation effects is evident from our study by the absence of spatial autocorrelation (Tab. 2, Fig. 3). In a scenario of precipitation effect, we should observe similarities in activity concentrations of isotopes between spatially close glaciers. Similarly, based on Pearson's correlation, Clason et al., (2023) also did not find a relation between the activity concentration of ^{137}Cs and the distance from the Chernobyl in the Northern Hemisphere. This suggests that in the case of ^{137}Cs and ^{241}Am, factors related to glacier features are more important for predicting current activity concentrations than the sources, distance and potentially received amounts of radionuclides in past. Therefore, the observed activity concentrations probably reflect the ability of specific glaciers to concentrate fallout radioisotopes more efficiently than others.

Table 1. Results of linear models (censored regression for ^{241}Am). *log-scaled vectors. P-values under 0.05 are bolded. For the censored regression, the "log(sigma)" refers to the proportion of unexplained variation by fixed effects, in this model 8 of 19 observations were left-censored. Comparison of models are presented in the Supplementary Table 4. Model summary for ^{210}Pb: adj. $R^2 = 0.83$, $F_{3, 15} = 29.82$, $P < 0.001$; ^{137}Cs: adj. $R^2 = 0.32$, $F_{2,16} = 5.28$, df = 16, P = 0.017.

Response	Parameter	Estimate	SE	t-value	p
^{210}Pb*	Intercept	10.727	1.2965	8.274	**< 0.001**
	Organic matter content	0.1437	0.0172	8.364	**< 0.001**
	Average altitude	-0.001	0.0004	-2.708	**0.016**
	Surface area*	-0.125	0.0548	-2.283	**0.038**
^{137}Cs*	Intercept	5.974	0.761	7.856	**< 0.001**
	Organic matter content	0.189	0.075	2.530	**0.026**
	Surface area*	-0.522	0.237	-2.209	**0.042**
^{241}Am*	Intercept	29.505	21.4497	1.376	0.169
	Organic matter content	-0.076	0.1398	-0.540	0.589
	Surface area*	-1.101	0.4823	-2.282	**0.022**
	Precipitation GF*	-3.328	2.7216	-1.223	0.221
	log(sigma)	*0.720*	*0.2384*	*3.019*	***0.003***

We observed a negative relation between the activity concentration of ^{210}Pb in cryoconite and the average altitude of a glacier (F = 1.495, df = 1, p = 0.039; Tab. 1, Fig. 4). The natural isotope ^{210}Pb on glaciers precipitates primarily as a product of ^{222}Rn decay, with short-lived nuclides between them in a decay chain. The unsupported fraction of ^{210}Pb is a majority in alpine cryoconite, consisting of 99.1% of total ^{210}Pb for Forni Glacier and 97.2% on Morteratsch Glacier (Baccolo, Łokas et al., 2020). As ^{222}Rn is a noble gas with a short half-life ($t_{½}$ = 3.6 days) originating from uranium-reach ores underground, its concentrations in the air decrease with the altitude (Baldoncini et al., 2017; Feichter & Crutzen, 1990), thus potentially explaining our observed negative relation with altitude. ^{210}Pb, an indirect daughter isotope (with other short-lived isotopes between), also follows this pattern linearly with altitude, as found in our case for cryoconite (Piliposian & Appleby, 2003). Results obtained in this study are not consistent with the reports of Clason et al., (2023), who found a positive correlation between altitude and ^{210}Pb activity concentrations in the Northern Hemisphere, while no other predictors were taken into account in that analysis (based on Pearson's correlation only). These studies differ also by the scale. Data from Clason et al., (2023) was collected globally, covering two groups of glaciers: the low-elevation glaciers in polar regions and high-elevation mountain glaciers. The low-elevated polar glaciers are located close to the seas, while the mountain glaciers are mainly on the continents. ^{222}Rn varies not only altitudinal but also between lands and oceans; the estimated contribution of the oceans in global ^{222}Rn release was lower than 5%, being significantly higher above grounds than the oceans (Baldoncini et al., 2017; Wilkening & Clements, 1975). Therefore, the results of Clason et al., (2023) show variation between altitudes, which could also be interpreted as the variation between two types of glacier localities. Indeed, the authors observed an effect of the distance from the ocean on ^{210}Pb activity concentrations. However, this variation was not included in their models, while, according to the results of PCA in that study, distance from the ocean was also positively correlated with mean elevation. It is possible that the observed effects were the results of collinearity. In this study, we controlled for this variation by analysing glaciers in one region, and we assessed a lack of spatial autocorrelation in ^{137}Cs, ^{241}Am and ^{210}Pb activities concentrations (Tab. 2; Fig. 3). This suggests a lack of local effects related to ^{222}Rn sources on ^{210}Pb activity concentrations. It is worth mentioning that even if ^{222}Rn shows a general trend of decrease with altitude on regional scales (Feichter & Crutzen, 1990), the activity concentrations of ^{210}Pb do not always follow this trend (Bourcier et al., 2011; Rastogi & Sarin, 2008). This might be due to the influence of local wind circulation, foehn wind, random wind gusts and the concentration of aerosols on which ^{210}Pb quickly adsorbs (Isakar et al., 2015). Moreover, it was shown that the ground-level of ^{210}Pb activity concentration should show some correlation with rain and snow data for the same region (Sykora & Froehlich, 2009). However, glaciers located closer which are subject to similar precipitations had no similar ^{210}Pb activity concentrations (Tab. 2; Fig. 3). It cannot be excluded that the observed increase of ^{210}Pb activity concentrations on highly elevated glaciers might be related to other factors not related to glacier size, organic matter content, or geographical position.

Table 2. Results of spatial autocorrelation analysis based on Moran’s I test.

Isotope	Moran's I statistic	Z	P
^{210}Pb	0.062	0.432	0.433
^{137}Cs	0.098	0.462	0.315
^{241}Am	0.147	0.512	0.184

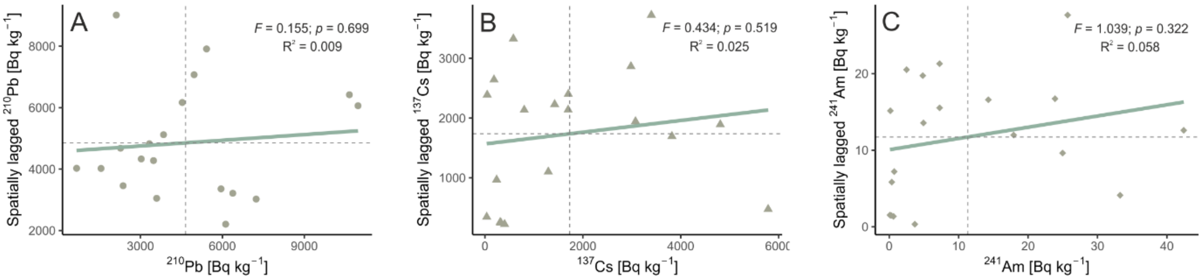


Figure 3. Lack of spatial autocorrelation of ^{210}Pb (A), ^{137}Cs (B) and ^{241}Am (C) activity concentrations. Dots represent observations for glaciers, a solid-coloured line is a regression line while dashed horizontal and vertical lines are means of observations (x-axis) and spatially lagged observations (y-axis).

The activity concentration of ^{210}Pb was positively related to the average organic matter content in cryoconite (F = 69.962, df = 1, p < 0.001; Tab. 1, Fig. 4). This inter-glacial relation is consistent with those observed in previous studies relating ^{210}Pb concentrations to OM contents of individual cryoconite samples within single glaciers (Buda et al., 2024; Clason et al., 2023). It was well-documented that negatively charged bivalent ^{210}Pb binds effectively to organic matter in various habitats (El-Daoushy & Garcia-Tenorio, 1988; Mihailović et al., 2014). The activity concentration of ^{137}Cs was also positively related to the organic matter content of cryoconite (F = 6.4, df = 1, p = 0.022; Tab. 1, Fig. 5), but this relationship was weaker, and the estimate error was higher (Tab. 1, Fig. 4 and 5). This aligns with the results of Buda et al., (2024) and Davidson et al., (2023), who, based on different approaches, confirmed that a significant proportion of ^{137}Cs in cryoconite is bound to mineral fractions rather than to organic matter itself, probably with mineral structures, while ^{210}Pb binds more strongly with the organic matter surfaces. This finding is significant as it suggests that the OM-minerals relationship may also influence the relationship between ^{137}Cs and OM in cryoconite. For instance, Buda et al., (2024) observed that cryoconite with higher OM is also characterised by a smaller fraction of minerals, leading to a larger surface area of minerals. Moreover, for ^{241}Am, the organic matter content was not related to its activity concentration (F = 0.292, df = 1, p = 0.598; Tab. 1). Overall, the importance of organic matter in binding radioisotopes depends on the chemical properties of the radioisotope. However, as the measurement of organic matter is straightforward and the explained variation is relatively large, it is a good predictor of the activity concentration of some radionuclides on glaciers.

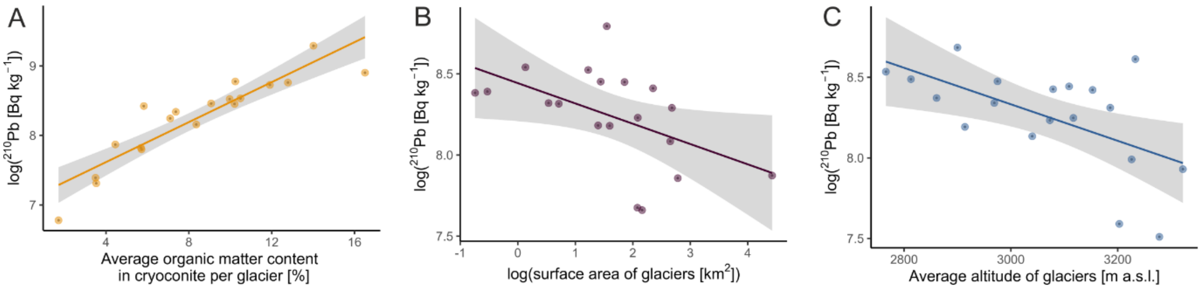


Figure 4. Relationship between ^{210}Pb activity concentration (log-scaled) and glacier-specific variables such as organic matter content in cryoconite (A), surface area of glaciers (B), and average altitude of a glacier (C). Plots represent selected model visualisation with partial residuals (dots). Therefore, each graph shows the relationship between a given independent variable and the ^{210}Pb while accounting for the effects of the other independent variables in the model.

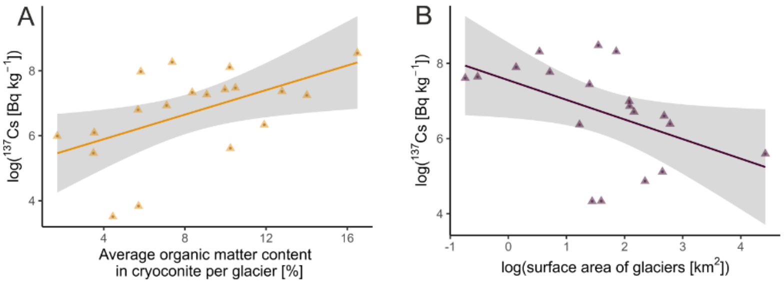


Figure 5. Relationship between ^{137}Cs activity concentration (log-scaled) and glacier-specific variables such as organic matter content in cryoconite (A) and surface area of glaciers (B). Plots represent selected models visualisation with partial residuals (dots). Therefore, each graph shows the relationship between a given independent variable and the ^{210}Pb while accounting for the effects of the other independent variables in the model.

Cryoconite on small disappearing glaciers as hotspots of radioisotopes

Understanding the fate of pollutants stored on glaciers upon melting and which glaciers are the most contaminated is crucial for assessing potential threats to downstream ecosystems. In this study, we observed a negative relationship between the activity concentrations in cryoconite and the surface area of glaciers for all subject radionuclides (^{210}Pb: F = 5.21, df = 1, p = 0.037; ^{137}Cs: F = 4.878, df = 1, p = 0.042; ^{241}Am: F = 5.208, df = 1, p = 0.039; Tab. 1, Fig. 4 and 5). This relationship might be due to the concentration of radioisotopes by cryoconite through intense glacier melting in the past, as well as long-term deposition and accumulation of organic matter along with other components of cryoconite on the glacier surface. During ablation, the surface of a glacier becomes a dynamic habitat (Takeuchi et al., 2018; Zawierucha et al., 2019) where cryoconite is redistributed and can effectively capture radioisotopes (Buda et al., 2024; Łokas et al., 2016; Owens et al., 2019).

The observed high activity concentrations of radionuclides on smaller glaciers might be related to the release of radioisotopes during the intensive melting of accumulation zones in the last decades. Glaciers in the Alps, despite keeping the average altitude similar, their accumulation zone moved upward by about 240m since the 1960s and 1970s compared to 2009 (Carturan et al., 2013). This suggests that a significant amount of relatively young snow and firn from the lower parts of accumulation zones melted and released stored radioisotopes over this period. In the case of small glaciers, we could expect lower dilution of those radioisotopes than in the case of big glaciers. Another possible explanation is related to variation in surface ice age. Ice that melts currently in the ablation zone of bigger glaciers can be older than that in the ablation zone of smaller glaciers. Therefore, cryoconite on bigger glaciers in the last decades was in contact with ice that formed hundreds or thousands of years in the past, significantly before the nuclear era and the release of anthropogenic radioisotopes, but also far enough to decay ^{210}Pb ($t_{1/2}$ = 20.3 yrs.). In such a scenario, only the accumulation of radioactivity from the atmosphere affected the concentration of fallout radionuclides in cryoconite. This is valid also for small glaciers, where radioisotopes bound to cryoconite continuously from the atmosphere (^{210}Pb), however taking into account that the age of surface ice in the border between accumulation and ablation zones of smaller glaciers can be relatively young (even below 50 yrs.; Festi et al., 2021), progressive melting releases radioisotopes bound in the ice (^{210}Pb, ^{137}Cs and ^{241}Am) in the accumulation zone and currently released them and concentrated in the cryoconite. However, to fully understand these potential reasons, more detailed data about the age of surface ice in the cryoconite zone on different glaciers in the Alps is needed.

Globally, glaciers are melting rapidly, a trend that has accelerated since the end of the 19th century (Marta et al., 2021; Pörtner et al., 2019; Zemp et al., 2013). Anthropogenic radioisotopes accumulated in large glaciers, especially those with a half-life too short compared to the persistence of ice on a glacier, do not pose a real threat to downstream ecosystems due to radioactivity. Our results show that many anthropogenic radioisotopes persist on glaciers and concentrate there during melting. This is consistent with Cao et al., (2023) and Clason et al., (2021), who demonstrated a high disproportion in the concentrations of radioisotope activities on glaciers compared to downstream habitats. Therefore, when considering large glaciers, we could observe a decrease in the radioactivity of these short-lived radionuclides before the ice melts. However, we believe that small glaciers, which are the most numerous in low and mid-latitude mountains and are highly threatened with disappearance (Huss & Fischer, 2016), are a special case. As radionuclides concentrate in the supraglacial sediment as melting progresses (Fig. 4 and 5), we could observe a relatively high peak of released radioactivity at the complete melt of glaciers. This is possible even for short-lived radionuclides, as around half of the smallest glaciers in the Swiss Alps are expected to melt within the next 25 years (Huss & Fischer, 2016), which is within the range of the half-life of the radioisotopes considered in this study. Such a scenario also seems to hold for Scandinavian glaciers that store a high amount of radioisotopes (Clason et al., 2021; Łokas et al., 2022) and 98% of them are predicted to disappear by 2100 (Nesje et al., 2008). On the one hand, ^{210}Pb - natural isotopes with constant deposition - will concentrate on glaciers during melting and then be released to downstream ecosystems from glaciers. A current comparison of activity concentration of fallout radionuclides shows that still some share of radioisotopes passes through glacier and reaches the proglacial zone, suggesting downstream accumulation of them with localised off-ice hotspots (Clason et al., 2021; Łokas et al., 2017). A study in British Columbia, Canada Owens et al., (2019) documented declining fallout radionuclides in fluvial sediments with increasing distance from the glacier terminus, while studies in Arctic Sweden (Clason et al., 2021) and Svalbard (Łokas et al., 2017) found that some radionuclides were higher in sediments from southern proglacial outlets than those from northern outlets, highlighting the importance of local variability in glacier topography and hydrology. However, given that a significant proportion of radioisotopes such as ^{137}Cs is bound to minerals (Buda et al., 2024), their mobility in downstream ecosystems might be limited, posing a real threat to glacier biota and strictly glacier-adjacent ecosystems which should be extensively monitored during ongoing glaciers melting. Moreover, already reported activity concentrations of radioisotopes in proglacial sediments also suggest that the sediment from glaciers might be diluted by the debris, lowering the negative effect on biota.

Following Beard et al., (2022), we emphasise the importance of monitoring radioactive contamination on glaciers and glacier-adjacent ecosystems as the radiative pressure seems to increase in cryoconite and glaciers melting. In our perspective, a particular case is mountain glaciers. On the one side, their location is related to the past high accumulation of anthropogenic radioisotopes. However, on the other hand, they terminate on the lands, providing water for people living down in valleys. Knowing that mountain glaciers in the northern hemisphere (Clason et al., 2023) show the highest radioactivity levels, we underline the need to monitor radioactivity pressure on glaciers and downstream ecosystems in the Alps, the Caucasus, central-Asian and North American mountain ranges.

Conclusion

The discussion on the glacier-related pollution increases in the literature. However, the debate mainly focuses on the direct measurements of the contaminants on glaciers, while the anticipation of hazardous glacial hotspots still needs to be included. In the context of intensive glacier melting, assessing the potential threats of stored pollutants to understand their impact on downstream ecosystems is crucial. Our comprehensive sampling across the Alps has demonstrated that glacier features such as surface area, altitude (^{210}Pb), and the amount of OM in the cryoconite play a more significant role in the accumulation of radioisotopes than geographic factors. Apart from the organic matter content in cryoconite and elevation (^{210}Pb), the surface area emerges as the most critical predictor of radioisotope activity concentrations on Alpine glaciers. The activity concentration of both natural (^{210}Pb) and anthropogenic (^{137}Cs, ^{241}Am) radioisotopes increases as the surface area decreases. This suggests that a significant proportion of radioactivity is bound to the supraglacial sediment, known as cryoconite, during the melting of ice, which contains natural and anthropogenic fallout radionuclides. This might be important, as the small glaciers in the Alps are predicted to disappear within the next 50 years, releasing stored radioisotopes. If so, we could witness intense abiotic pressure on glacier-adjacent ecosystems as glaciers disappear. Multidimensional studies are required to understand the potential effects of increasing radioactivity, seemingly linked to ongoing glacier melting.

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CHAPTER III

Exploring radioactivity-biodiversity relationships in the supraglacial ecosystem

Abstract

Considering the previous results, next step of the investigation was devoted to understanding the relationships between elevated environmental radioactivity and microbial diversity and the abundance of top consumers (Tardigrada) in cryoconite. Microbial richness was assessed based on DNA amplicon sequencing of 16S V4 rDNA and 18S V9 rDNA from 16 glaciers collected in the same year. This lowered the effects of known temporal variation in microbial community composition on glaciers. In the case of top consumers abundance it was 19 glacier collected over two years. Activity concentrations of anthropogenic (^{137}Cs) fallout radionuclide ranged in mean for glacier from 36 Bq kg^{-1} to 5 785 Bq kg^{-1}, while for the natural one (^{210}Pb) it was from 1 013 to 10 711 Bq kg^{-1}. Such high variation was reflected in microbial diversity, which decreased as radioactivity increased. In the case of bacteria, the negative relation between radionuclides activity concentration and richness (χ^2 = 1.06, df = 1, p = 0.002) is similar for both nuclides (χ^2 = 9.69, df = 1, p = 0.303). However, in the case of micro eukaryotic communities, the effect was also negative (χ^2 = 3.41, df = 1, p = 0.065), but lower than in the case of bacteria, and was slightly stronger for ^{210}Pb than for ^{137}Cs (χ^2 = 3.34, df = 1, p = 0.068). These results are supported by the risk assessment in the previous chapters, where ^{210}Pb is more strongly associated with organic matter and organisms itself, while ^{137}Cs with mineral fractions. Contrary to expectations that IR affect top consumers, not directly, but as a response to ecosystem functioning changes related to microbial community loss, abundance of tardigrades on glaciers was not related to activity concentrations of both nuclides (χ^2 = 0.001, df = 1, p = 0.979). This work provides the first piece of evidence that elevated concentration of radioisotopes on glaciers can be a threat to biodiversity and ecosystem function. Knowing that elevated radioactivity in the Alpine glaciers impacts microbial communities, there is a high need to monitor radioactivity pressure on glaciers and downstream ecosystems in the Alps, but also in the other regions with reported high activity concentrations of fallout radionuclides such as the Caucasus, central-Asian and north-American mountain ranges.

Introduction

Ionizing radiation (IR) occurs on the Earth naturally as cosmic radiation that penetrates the atmosphere and in a significant amount due to decays of primordial and secondary radioisotopes, mainly uranium and thorium decay chain products. Even though IR is common, the knowledge of the effects of chronic radiation on the organisms in their natural environments, especially the most diverse and abundant microscopic prokaryotes and eukaryotes, is missing. Radioisotopes are widely spread over our globe in the Earth's mantle and the atmosphere (El-Daoushy & Garcia-Tenorio, 1988; Mihailović et al., 2014; Rastogi & Sarin, 2008; Trieloff & Kunz, 2005). During the last century, the Earth received another source of radioisotopes due to human actions related to nuclear weapons and nuclear energy that resulted in the release of anthropogenic radioisotopes such as ^{90}Sr, ^{137}Cs, $^{238\text{-}240}$Pu or ^{241}Am (Bunzl & Kracke, 1988; Evrard et al., 2020; Ikeda-Ohno et al., 2016; Werner & Purvis-Roberts, 2007). Above 2000 nuclear tests have been done since 1945, of which more than 500 blasted in the atmosphere, spreading radioisotopes over the globe (Mietelski, 2010). Moreover, Nuclear Power Plant (NPP) accidents, satellite accidents equipped with radioisotope thermoelectric generators, and other accidents related to nuclear weapons transport or processing of nuclear fuel contributed locally or globally to the anthropogenic radioisotope contamination. Released high amounts of radioisotopes contain these elements which do not occur in nature or only in trace concentrations (e.g., ^{244}Pu, ^{240}Pu, ^{239}Pu), but also those that widely occur naturally, but their amount since 1945 was elevated, for instance, ^{3}H or ^{14}C (Hodge et al., 2000; Mietelski, 2010).

Numerous studies, including laboratory experiments and in-situ observations, have analysed the effects of low-dose chronic IR on organisms. In the contaminated area close to Chernobyl NPP negative effects of chronic radiation on people's health were observed, specifically the increased frequency of proliferative atypical cystitis (Romanenko et al., 2009), thyroid cancers or leukaemia (Moysich et al., 2002). In the same area, IR affects small rodent's lipid metabolism, immune system, gut health and gut microbiota (Jernfors et al., 2024; Kesäniemi et al., 2019). Moreover, increased genetic diversity was observed in *Pinus sylvestris* along the exposure gradient close to Chernobyl NPP (Geras’kin & Volkova, 2014), but also cytogenetic and phytohormonal changes in *Pinus resinosa* in Fukushima prefecture (Geras’kin et al., 2021). Despite all these evidences, the information on how radioactivity due to global fallout can influence organisms far from test sites and areas of NPP disasters is missing.

A relatively high concentration of fallout anthropogenic radionuclides but also natural ones were found on glaciers across the planet (Buda et al., 2020; Clason et al., 2023; Łokas et al., 2018, 2022; Pinglot et al., 1994). Fallout radioisotopes are effectively captured from the surface glacial and snow melt-water by dark sediment redistributed on the ablation zone on glaciers (Baccolo et al., 2017; Buda et al., 2024; Clason et al., 2023; Łokas et al., 2022; Owens et al., 2023). This sediment, called cryoconite, is a biological hotspot on glaciers worldwide, during summer it can melt into the ice creating water-filled depressions called cryoconite holes (Fig. 1). Activity concentrations of natural and anthropogenic radioisotopes in cryoconite are by a magnitude higher than in surrounding habitats (Baccolo, Łokas, et al., 2020; Clason et al., 2021; Łokas et al., 2017; Owens et al., 2023). Organic matter (OM) content is the most important predictor for the accumulation of radioisotopes (^{137}Cs, ^{210}Pb, $^{239+240}$Pu) in the cryoconite (Baccolo et al., 2017; Buda et al., 2024; Clason et al., 2023; Łokas et al., 2022). However, OM does not fully explain the cause-effect relationships, as analysis of radioisotopes mobility in the Alpine cryoconite has shown that even if the ^{137}Cs is positively related to organic matter content, their significant fraction (77%) is firmly bound to the minerals, while for ^{210}Pb less than 10% (Buda et al., 2024). A similar conclusion was drawn using a different approach on Icelandic glaciers (Davidson et al., 2023). However, even if some of the fallout radioisotopes are not directly related to organic-metallic complexes, they have to pose a threat to organisms, as they are present in the consumers (springtails) that live on the surface of glaciers in the Alps and feed on biofilms of supraglacial debris (Buda et al., 2024; Jaroměřská et al., 2023). The uptake of radioisotopes from sediment by these arthropods varied from 0.07 to 0.117, with relatively low variation between radioisotopes (^{137}Cs, ^{210}Pb and $^{239+240}$Pu). Moreover, even if a significant fraction of radioisotopes is bound to the minerals' surface, not to the organic itself, they can still interact with organisms as microbial communities form biofilms on the mineral grains' surfaces, potentially affected by electrons emitted by ^{137}Cs decays (Buda et al., 2024).

The biological activity of cryoconite represents diverse metabolic and taxonomic groups, with the domination of heterotrophic and autotrophic bacteria, fungi, algae and representants of protozoa (Cameron et al., 2012; Franzetti et al., 2017; Pittino et al., 2018; Pittino, Zawierucha, et al., 2023; Xu et al., 2010; Zawierucha et al., 2018). The microbial communities differ between regions, glaciers and even cryoconite holes (Buda et al., 2022; Mueller et al., 2001; Pittino, Ambrosini, et al., 2023). The biomass of photoautotrophic organisms varies between glaciers and time, with the dominance of green algae and Cyanobacteria (Buda et al., 2022; Franzetti et al., 2017; Pittino et al., 2018). The Cyanobacteria play the role of cryoconite ecosystem engineers by producing extracellular polymeric substances, which stick together with other organisms and minerals, but also potentially bind radionuclides (Takeuchi et al., 2010; Wejnerowski et al., 2023). Such aggregates show spatial variation of metabolic groups with more aerobic in the surface and anaerobes in the inner layers (Poniecka et al., 2018; Segawa et al., 2020). Top consumers in this ecosystem are micro invertebrates such as tardigrades and rotifers that occupy different feeding niches due to their different foraging strategies (Jaroměřská et al., 2021; Zawierucha et al., 2021). Their relative abundance varies between glaciers and regions, while in the Alps, tardigrades dominate (Zawierucha et al., 2021). The relatively simple trophic web in cryoconite, relatively high concentrations of fallout radionuclides that excess surrounding habitats (Baccolo, Łokas, et al., 2020; Clason et al., 2021), control of biota primarily by physical (hydrological and meteorological) factors and patchy distribution of cryoconite holes make them a suitable model for testing the relation between low-dose chronic environmental IR and organisms.

The main aim of this chapter is to parameterise whether the pollution of glaciers represented by natural and anthropogenic radioactivity is elevated enough to affect population-level organisms inhabiting biological hotspots on glaciers – cryoconite holes. The study was conducted in the Alps, where previous studies have shown elevated radioactivity of fallout radionuclides (Baccolo, Nastasi, et al., 2020; Buda et al., 2024; Wilflinger et al., 2018), but also presented that organisms are differently related with radioisotopes (^{137}Cs and ^{210}Pb) what gives the background of experimental design of this study. Considering that the surface of alpine glaciers is a dynamic ecosystem (McIntyre, 2011), where the distribution of organisms changes constantly due to intensive ablation (Zawierucha, Buda, & Nawrot, 2019), analysis was performed between glaciers, not on a glacier scale. The main hypothesis is that due to the negative effects of IR, biodiversity measured as bacterial and eukaryotic community richness based on DNA amplicon sequencing is decreasing as IR increases. To understand this relation in the context of the most important environmental parameters, two additional variables which can affect biodiversity the most were selected. According to biogeographical island theory

(MacArthur & Wilson, 1967), higher diversity should be observed on bigger islands (glaciers). Moreover, the vegetation period (time during summer without snow cover in the ablation zone) shortens with the altitude of the glacier. Those glaciers located higher are longer isolated and in longer distance from other habitats. Moreover, increasing altitude also results in increasing UV radiation. These three altitude-related potential pathways in affecting diversity are not mutually exclusive but likely negative. The cryoconite holes on the glaciers in the Alps are dominated by one group of top consumers – tardigrades. This allows to assess whether disturbances in ecosystems in lower-trophic groups can be visible by the change in the population structure of top consumers in cryoconite. Knowing that the microbial community can change over the years (Pittino et al., 2018), only samples collected in the same summer season from 16 glaciers were used. On the other hand, the community of tardigrades remains relatively stable on a glacial scale between seasons (Zawierucha, Buda, Azzoni, et al., 2019). Therefore, cryoconite collected over two constitutive years was used in this study for further analysis. This study first provides insight into understanding whether elevated natural and anthropogenic radioactivity can impact organisms on glaciers and presents community analysis based on broad sampling during one year in one region – the Alps.

Methods

Sampling

In total, 19 glaciers were sampled in the Alps in the range from Dauphiné to the Central Eastern Alps during the summer season in 2020 and 2021 (Fig. 1). On each glacier, 8-10 samples of cryoconite from cryoconite holes were collected with clean stainless steel spoon or plastic pipette to sterile test tubes or bags. The material was frozen at -20°C or conserved with 96% ethyl alcohol and then kept at -20°C as soon as possible. Knowing that the distribution of top consumers is heterogeneous at the cryoconite hole's bottom (Zawierucha, Buda, Fontaneto, et al., 2019), the sediment was sampled multiple times from the bottom of the holes. Before further analysis, the material was mixed and split into separate analyses. The surface area of glaciers, their average and maximum altitude were estimated for 2015/16, with one extent due to lack of data for 2015/16 (2022, Pers glacier), based on the Global Land Ice Measurements from Space (GLIMS) database (Raup et al., 2007). In total, samples from 16 glaciers collected in one year were used for microbial diversity analysis, while 19 glaciers collected over two years were used for top consumer analysis.

Radiometric analysis

The activity concentrations of ^{137}Cs and ^{210}Pb were measured using a low-background, digital gamma-ray spectrometer equipped with a Broad Energy Germanium (BEGe) detector BE5030 with a relative efficiency of about 48% and multilayer passive shield surrounded by an active shield's detector (Gorzkiewicz et al., 2019). ^{137}Cs activity was determined by measuring the 661.6 keV emission peak of ^{137m}Ba, while for ^{210}Pb, the 46.5 keV peak was used as an analytical signal. Efficiency calibration, including self-absorption correction, for used measurement geometry was determined using LabSOCS calibration software (Mirion Technologies). The spectra were collected over approximately 24 hours, or in case of highly active samples, it was 12 hours. The activity concentrations were corrected on the day of sampling. Data quality was evaluated by measuring of IAEA Reference Materials (IAEA 447).

Biodiversity assessment and top-consumers abundance estimation

Total DNA from sediment was extracted with FastDNA SPIN Kit for Soil from about 0.5 ml of wet cryoconite following the manufacturer's instruction. Hypervariable V4 16S rDNA and V9 18S rDNA regions were used to assess bacterial and eukaryotic community structures. Primers were tailed at 5′ ends with dual-indexed IonTorrent adapters for sequencing using the Ion Torrent system (Life Technologies, USA). Primer pairs were used following Zawierucha et al., (2022). The focal regions were amplified in two technical replications. Each PCR was carried in 10 µl containing Hot FIREPol DNA Polymerase (Solis BioDyne, Tartu, Estonia), 0.25 µM of each indexed primer and 1 µl of DNA template. Amplification started with preliminary denaturation at 95°C for 12 minutes, then followed by 35 cycles of 15 s at 95˚C, 30 s at 50˚C and 30 s at 72˚C, with a final extension step at 72˚C for 5 min. A blank sample was prepared for each DNA isolation kit and PCR similarly. The efficiency of amplification was tested on an agarose gel. Amplicons corresponding to genetic markers were pooled, agarose gel-fractionated, and purified using 3% agarose gel electrophoresis and QIAquick Gel Extraction Kit (Qiagen, Germany) according to the manufacturer's instructions. The concentration and amplicon length distribution of libraries were checked using High Sensitivity D1000 Screen Tape assay on a 2200 Tape Station system (Life Technologies, USA). Sequencing was carried out using Hi-Q View Sequencing Kit and Ion S5 system on an Ion 540 chip (Life Technologies, USA) according to the manufacturer's instructions. amples with low raw reads (< 5 000) were resequenced.

All tardigrade specimens were identified by 18S rDNA V9 amplicons from eDNA. For abundance analysis, individuals were isolated from the sediment under stereoscope microscopy on Petri dishes and then counted. The volume of sediment was measured prior to tardigrades isolation and used in further analysis as an offset.

Data analysis

Raw sequence were pre-filtered by Ion Torrent Suite software version 5.10.1 (Life Technologies, USA) to remove polyclonal and low-quality sequences. The quality of sequences was asses by FastQC and MultiQC software after removing 5′ and 3′ primers. Sequences were clustered in Amplicon Sequence Variants (ASVs) using DADA2 (Callahan et al., 2016). Chimeras, which contained an average 11.09% of filtered reads for 16S V4 and 1.76% of 18S V9 reads, were discarded from further analysis. Detailed procedure, the full track of reads, and the error rate model visualisations are presented in the repository linked at the end of this section. Finally, the ASVs were taxonomically assigned to SILVA (16S rDNA V4 – release 138.1; 18S rDNA V9 – release 128) databases. For eukaryotes, all reads belonging to Fungi were removed because the performance of the used primers pair was not validated for fungal composition analysis, moreover reads belonging to Arthropoda and Nematoda were also excluded as the false observation (each sample was scanned under a stereoscope microscope for metazoan composition and compare with DNA reads).

Relative abundance of 16S V4 and 18S V9 rDNA high-rank taxa were calculated to visualise interglacial variation. To understand whether biodiversity can be shaped by elevated radioactivity, the number of ASV in a sample was used as a proxy of richness calculated using Phyloseq package (McMurdie & Holmes, 2013). The sequencing depth was enough to represent diversity in the samples, based on rarefaction curves, therefore to avoid error-rate inflation the reads data were not normalized. Due to their relatively short length of 18S V9 rDNA amplicons (100-130 bp), most ASVs were not classified with the required confidence score (80) for the Naïve Bayesian Classifier, therefore, they were classified with a minimum bootstrap of 50. This approach let to avoid removing numerous of ASVs, while the threshold of 50 is still informative for short sequences

(Wang et al., 2007). However, as the primary goal was to analyse the change in diversity across the environmental radioactivity based on ASV numbers, the analysis proceeded with this data.

The Negative Binomial (quadratic parametrization) Generalized Linear Models (GLMs) were built separately for bacterial and eukaryotic communities to test whether the elevated environmental radioactivity can reduce diversity in the cryoconite. These models contained the number of ASV as the response variable, the total activity concentration (sum of ^{137}Cs and ^{210}Pb), the interaction between total activity concentration and ratio of ^{210}Pb to ^{137}Cs, and the surface area as well as the altitude of the glacier as fixed effects. This structure led to testing the overall impact of elevated radioactivity on microbial communities, keeping information about the differences between the effects of ^{210}Pb and ^{137}Cs by including interaction terms. Moreover, to test whether the abundance of the top consumers in cryoconite can be influenced by elevated environmental radioactivity, a GLM that contained the number of individuals as the response variable, the total activity concentration (sum of ^{137}Cs and ^{210}Pb), the interaction between total activity concentration and ratio of ^{210}Pb to ^{137}Cs as fixed effects and the volume of sediment that was used for top consumers abundance estimation as the log-scaled offset. Before modelling, each variable was averaged per glacier level. The model estimates was obtained using Restricted Maximum Likelihood Estimation. The overall significance of fixed effects were estimated based on type-III Anova using likelihood-ratio chi-square tests.

All models were implemented in R 4.3.2 (R Core Team, 2024) using build-in functions and glmmTMB package (Brooks et al., 2017) while checking for any violation of assumption based on diagnostic plots using Performance package (Lüdecke et al., 2021). All raw demultiplexed sequence data, including negative controls, are deposited in SRA under PRJNA1091145, while the metadata related to samples as well as data analysis project are provided in the repository under the link: https://github.com/jakbud1/Chapter-III.git.

Results and discussion

Variation of ^{137}Cs, ^{210}Pb and covariates

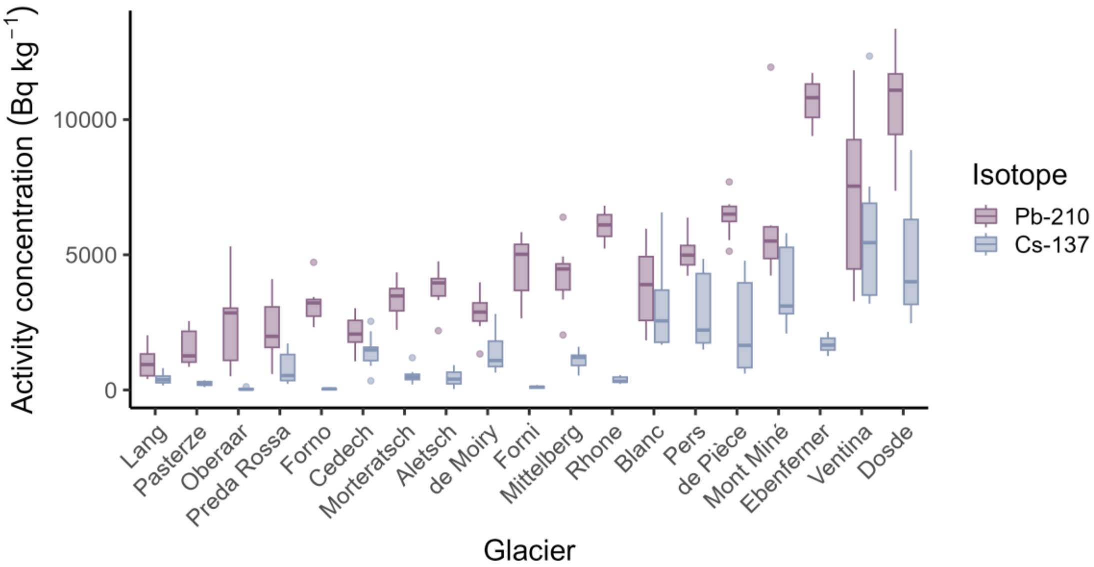

Figure 1. Intra- and interglacial variation of ^{137}Cs and ^{210}Pb activity concentration on glaciers, measured using gamma spectrometer with BEGe detector. Glaciers are ordered from the lowest total activity concentrations to the highest (^{137}Cs and ^{210}Pb activity concentration).

Activity concentration of natural (^{210}Pb) and anthropogenic (^{137}Cs) radioisotopes significantly varied between analysed glaciers (Fig. 1). The highest activity concentrations of ^{137}Cs were observed for the glacier Ventina (mean = 5785 Bq kg^{-1}) and the lowest on the glacier Oberaar (mean = 36 Bq kg^{-1}). The highest activity of ^{210}Pb was observed on the glacier Ebenferner (median = 10 711 Bq kg^{-1}), while the lowest was on the glacier Lang (mean = 1013 Bq kg^{-1}). These values align with previously reported activity concentrations in the Alps (Baccolo, Nastasi, et al., 2020; Wilflinger et al., 2018). The surface area of glaciers varied from 0.48 (glacier Preda Rossa) to 83.38 km^2 (glacier Aletsch), while the average altitude was from 2766 (glacier Forno) to 3322 (glacier Cedech) m a.s.l.

The richness of bacterial and eukaryotic communities varied strongly between glaciers, with different microbial community compositions based on high-level taxa relative abundance (Fig.2). The lowest number of Bacterial ASV was observed on glacier Mont Miné (61 ASVs), while the highest for glacier Lang (257 ASVs). On the other hand, the lowest number of ASV for eukaryotes was observed on the Morteratsch glacier (119 ASVs), while the highest was for the glacier Pasterze (397 ASVs). The total number of ASVs was higher for Bacteria than for Eukaryota (6943 vs 5715 ASVs). The number of bacterial ASVs was lower on 15 out of 16 glaciers. Both gene fragments of rDNA used for this assessment differ in length and evolution history. Therefore, a comparison in the number of ASVs altogether is not valid. Consequently, further analysis of bacteria and eukaryotes will be discussed separately.

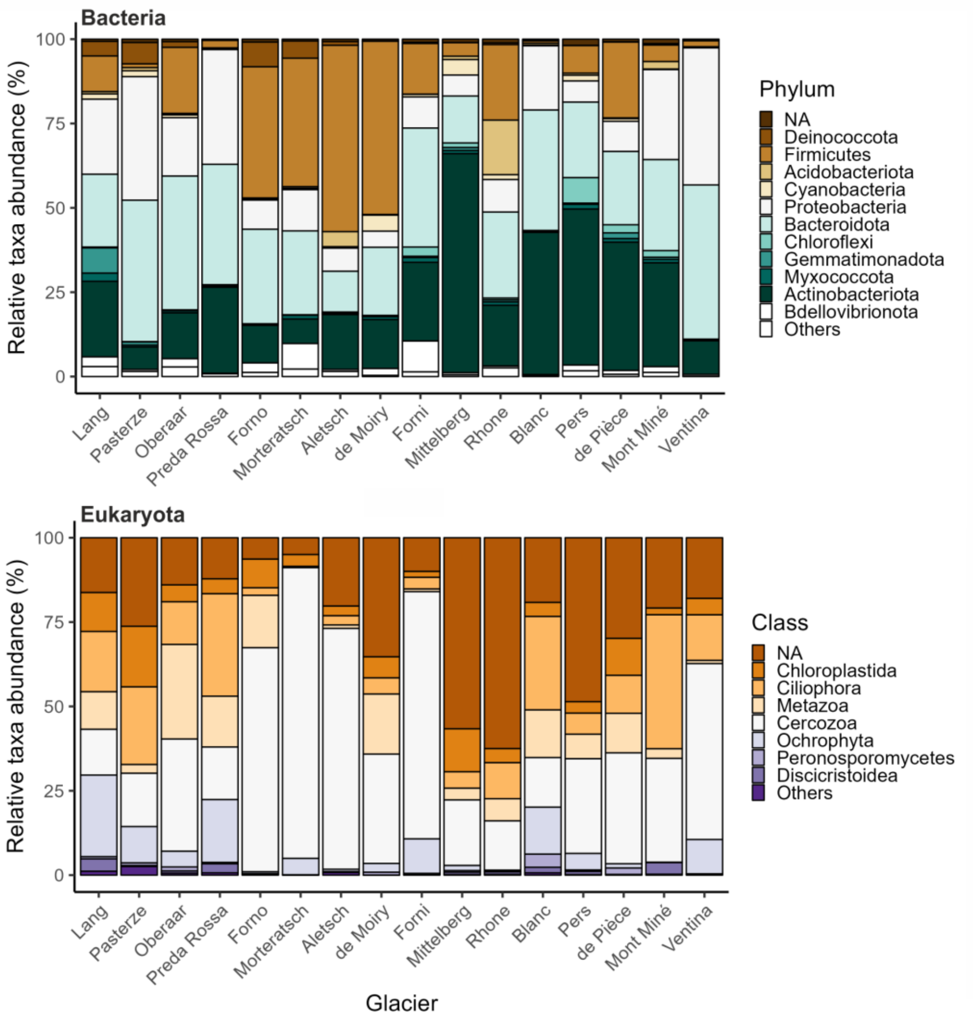

Figure 2. Bacterial (Phylum) and eukaryotic (Class) variation of high-taxa rank between glaciers. Taxa representing less than 1% of total abundance were grouped into the others group. Taxa were assigned using the Naïve Bayesian Classifier, with a minimum bootstrap of 80 for Bacteria and 50 for eukaryotes. Glaciers are ordered from the lowest total activity concentrations to the highest (^{137}Cs and ^{210}Pb activity concentration).

Relationship between environmental radioactivity and microbial diversity

Bacterial richness in cryoconite on glaciers was negatively related to the radioactivity measured as the sum of ^{210}Pb and ^{137}Cs activity concentrations ($\chi^2 = 1.06$, df = 1, p = 0.002; Fig. 3); this effect was not dependent on the ratio of ^{210}Pb to ^{137}Cs activity concentrations ($\chi^2 = 9.69$, df = 1, p = 0.303; Fig. 3). Control variables, the size and altitude of glaciers were not significantly related with bacterial richness in contrast to the total radioactivity (Tab. 1). This results suggests that IR emitted both ^{137}Cs and ^{210}Pb have similar negative effects on bacterial communities decreasing their biodiversity (Fig. 3). Bacterial communities in cryoconite can form biofilms on minerals, but also are related to decomposition of dead organic matter and cryoconite granules (Langford et al., 2010; Takeuchi et al., 2001; Zawierucha, Baccolo, et al., 2019). Previous results in the first chapter show ^{137}Cs are more strongly bound to mineral surfaces than to developing communities, in contrast to ^{210}Pb. Both ^{137}Cs and ^{210}Pb were related to organic matter in general. However, the photoautotrophic communities were more strongly associated with ^{210}Pb. Electrons emitted over the decay of ^{137}Cs can, therefore, affect bacterial communities forming biofilms on minerals, while the ^{210}Pb can affect bacteria interacting with organic matter. It is essential to point out here that these processes are not exclusive. Indeed, the first chapter shows that some fraction of ^{137}Cs is related to organisms, too, while the ^{210}Pb is to minerals. On the other hand, gamma quants of excited daughter radioisotopes seem not to affect organisms when considering their penetration to the scale we consider.

The richness of microeukaryotes in cryoconite tends to be negatively related to total activity concentration ($\chi^2 = 3.41$, df = 1, p = 0.065; Fig. 3), but this relation is slightly stronger, as the ratio of ^{210}Pb/^{137}Cs increases ($\chi^2 = 3.34$, df = 1, p = 0.068; Fig. 3). Although the results are above the commonly accepted significance level of 0.05, however, observed trend is in line with previous results and risk-related assessment in the first chapter. Moreover, these results are based on a robust approach with model parameters estimated using the Restricted Maximum Likelihood method. Overall, this suggests that in the case of microeukaryotes, ^{210}Pb has stronger effects than IR emitted by ^{137}Cs (Fig. 3). According to previous results of Chapter I and Davidson et al. (2023), ^{137}Cs are bound more strongly to minerals than ^{210}Pb. Microeukaryotes found in this study represent different ecological niches. Chloroplastida and Ochrophyta, being dominantly photoautotrophs, can be related to mineral surfaces and the organic compounds they stick to due to EPSs produced by bacteria. On the other hand, the taxa belonging to Ciliophora and Cercozoa constantly interact with organic matter, which is their source of food both as dead organic matter (^{137}Cs and ^{210}Pb), but also the photoautotrophic communities (bounds ^{210}Pb), not minerals themselves. This can explain that IR from ^{210}Pb, firmly bound to developing communities and dead organic matter in cryoconite, can affect microeukaryotes stronger than ^{137}Cs.

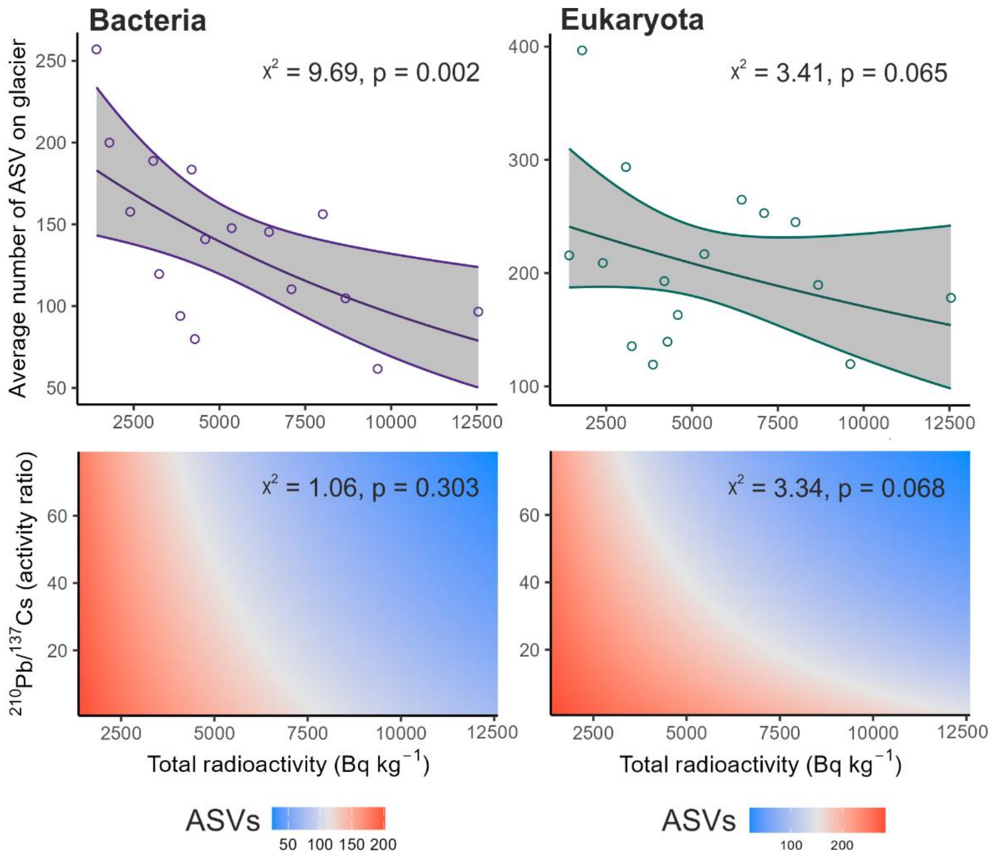

Figure 3. The negative relationship between environmental radioactivity (^{137}Cs and ^{210}Pb activity concentrations) and microbial diversity as the average number of ASV in cryoconite on glaciers. In all models, the altitude of glaciers and their size were included as control variables. The upper panels represent the effects of total activity concentration on microbial richness. In contrast, the lower panel shows effects with interaction of ^{210}Pb/^{137}Cs ratio, which shows the contribution of specific radionuclides in the observed negative relation. Models represent the results of GLMs with the tested significance of fixed effects using type-III Anova with likelihood-ratio chi-square tests.

Lacking relationship between environmental radioactivity and top-consumers abundance

The expectation was that IR could indirectly affect the abundance of top consumers by the response to ecosystem functioning changes related to microbial community loss or directly by IR related to food and animals. On the glacier with the highest total radioactivity (12 542 Bq kg^{-1}), 257 ASV of Bacteria and 216 ASV of eukaryotes were observed. In comparison, on the glacier with the lowest total radioactivity (1 418 Bq kg^{-1}), 96 ASV belong to Bacteria, and 178 ASV belong to eukaryotes. However, contrary to expectations, top consumers abundance does not follow this trend (χ^2 = 0.001, df = 1, p = 0.979; Fig. 4). According to results of amplicon sequencing, all presented 24 ASV belongs to *Cryobiotus klebelsbergi* with two dominant ASV which constitute 90.84% and 8.33% of relative abundance, while the 22 remaining ASV consist in total only 0.83%.

The average number of ASV in a sample is only 1.48 (SD = 0.94). Therefore, this data structure does not let to test whether the diversity of ASV is related to radioisotope activity concentrations.

The observed lack of effects of radioactivity on tardigrades might be because of the omnivorous diet of *C. klebelsbergi*, where likely the lack of food resources of one type of food is compensated by other types, which develops due to reduced competition between taxa. Rising evidence shows that the Alpine glacier-dwelling tardigrade, *C. klebelsbergi*, is not a food specialist but rather a food generalist (Jaroměřská et al., 2023; Zawierucha et al., 2022). Such a feeding strategy can be highly adaptive in fast-changing supraglacial ecosystems (Zawierucha, Buda, & Nawrot, 2019), where microbial communities can change significantly during and over seasons (Franzetti et al., 2017; Pittino et al., 2018). On the other hand, the variation between the main phyla of bacteria and the main phyla of eukaryotes is significant between glaciers. However, all high-level taxa are represented on all glaciers (Fig. 2). Results obtained in this study highlight a high need to understand trophic interaction in cryoconite, as they are crucial in the assessment of pollutant-related risk, which is high, not only from the FRNs but potentially due to all atmospheric-delivered pollutants.

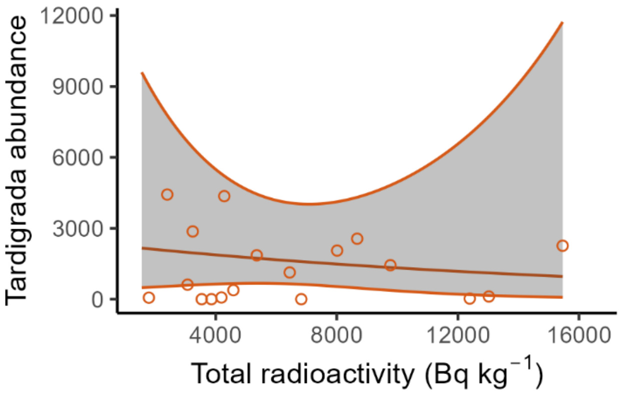

Figure 4. A lack of relationship between environmental radioactivity (^{137}Cs and ^{210}Pb activity concentrations) and the abundance of top consumers (Tardigrada) in the cryoconite on glaciers. The model represents the results of GLM with the tested significance of fixed effects using type-III Anova with likelihood-ratio chi-square test.

Significance of this study in understanding effects of IR on microbial communities

In the XX century, anthropogenic activities released radioisotopes spread globally. Studies related to the effects on biota were mainly limited to people's health and ecosystems closely related to NPP, such as Chernobyl and Fukushima Daichi (Geras'kin et al., 2021; Moysich et al., 2002; Nybakken et al., 2023; Steinhauser et al., 2014), but also to high contaminated areas such as Lake Karachay (Atamanyuk et al., 2012). However, even in high-risk areas, less attention was given to microbial communities. Assessment of IR effect on those microbial communities in natural conditions is crucial, as they act as ecosystem engineers, while changes in their composition are broad due to bottom-up control (Dobrovol'skaya et al., 2015; Saccá et al., 2017). For instance, long-term low-dose IR impacted microbial communities and resulted in a strong negative interaction in the carbon and nitrogen migration process among plants, microorganisms, and soil (Videvall et al., 2023). Analysing the effects of high-dose IR on microorganisms in controlled conditions has shown decreased survivorship due to broad metabolic dysfunctions (Reisz et al., 2014). However, the critical question is how they respond in natural conditions while the results are still scarce.

Results of Gu et al., (2014) have shown a decrease in bacteria richness but also abundance along with a gradient of radioactivity in the environment varying in total activity concentration of radioisotopes: 100 – 200 Bq kg^{-1}, 1 000 – 2 000 Bq kg^{-1} and over 10 000 Bq kg^{-1} in North-West China. These results are similar to those presented in this thesis, and what is important is that the scale of IR is similar. Another study, but with a mesocosms experimental approach, has shown that soil bacterial diversity decreases as a dose of IR increases; however, due to reduced competition, their functions are covered by fungi and algae species, which show increased diversity (Ogwu et al., 2019). On the other hand, the results of in situ observation in an area close to Chernobyl NPP (Videvall et al., 2023) have shown that elevated IR does not influence the diversity of microbial communities in aquatic habitats but can shape their distribution shown by variation in beta-diversity. The results obtained in this study for the first time show that elevated radioactivity from natural and anthropogenic sources can act as strong selective pressure on glacial microbial communities. Another step should follow the idea of understanding of the effects of IR not only on diversity per se but also on functional diversity. Moreover, there is a need to understand whether other atmospheric-delivered pollutants can impact organisms on glaciers and how they will respond to the melting of glaciers. Questions about the effects of pollutants stored in glaciers on the glacier and glacier-adjacent ecosystems remain still open, and systematic, coordinated monitoring of pollutants released by glaciers can be a key to understanding this problem globally.

Limitations of this study

Considering this study's correlative characteristic, there are some limitations in inference related to the effects of elevated IR on glacier surface biota. Recent work of Pittino, Zawierucha, et al., (2023) has shown that the alpha diversity of bacteria in cryoconite assessed on rDNA can be biased compared to rRNA. The DNA of dead organisms can persist in cryoconite, which is a cold (around 0°C) and anaerobic ecosystem (Buda et al., 2022; Poniecka et al., 2018). As a result, due to the accumulation of DNA in the sediment, this can mask low-abundance taxa that are active and detectable using rRNA amplicon sequencing.

Another major issue is that in this study, as a dependent variable, the activity concentration of radionuclides was used instead of the dose. However, currently, developed models do not allow the estimation of dose for such a complex spatial system related to microbial communities in soils when we operate on thousands of species interacting with each other with mineral fraction. Obtained data about the spatial relation of IR and organisms must be validated more to estimate dose, limited to the most abundant organisms. However, they help understand radionuclides' fate in glacial ecosystems and their potential relation to glacier and glacier-adjacent ecosystems.

The last important limitation is the lack of well-studied links between microeukaryotes and their ecological niches as a reference. Most studies on microorganisms in cryoconite holes focused on bacterial diversity, their physiological and metabolic variability, and spatial variation. However, in the case of microeukaryotes, studies were dominated by a focus on specific groups such as invertebrates and algae (mostly green algae), while those related to broad ecological connections were limited. This is probably due to logistical challenges in studying those interactions based on in situ glacier observations. On the other hand, the experiments are not easy to prepare, and mirroring specific glacial conditions in the laboratory is challenging. Understanding specific interactions of microeukaryotes in cryoconite could be beneficial in understanding the effects of specific radioisotopes and detailed consequences on ecological processes on the surface of glaciers.

Conclusion

This study, based on intensive sampling across 19 glaciers in the Alps, shows that microbial communities in cryoconite holes on the surface of glaciers are influenced by environmental radioactivity due to elevated activity concentrations of fallout radionuclides (^{137}Cs and ^{210}Pb). In the case of bacteria, the effect is similar for both nuclides, while in the case of eukaryotic communities, ^{210}Pb has a stronger effect. These results are supported by the risk assessment in the previous chapters, where ^{210}Pb is more strongly associated with organic matter and organisms itself, while ^{137}Cs with mineral fractions. This work provides the first piece of evidence that significant accumulation of radioisotopes by glacial biota can be a threat to the biodiversity and population survivability on glaciers. However, we still need to deepen our understanding of this process. Knowing that the DNA richness estimates can be biased in cold habitats (Pittino, Zawierucha, et al., 2023), other analyses should be taken into account when analysing the effects of IR on organisms, as those using different approaches, including controlled experiments and RNA-based richness estimation or metabolic pathways and functional diversity analysis.

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Supplementary materials – Chapter 1

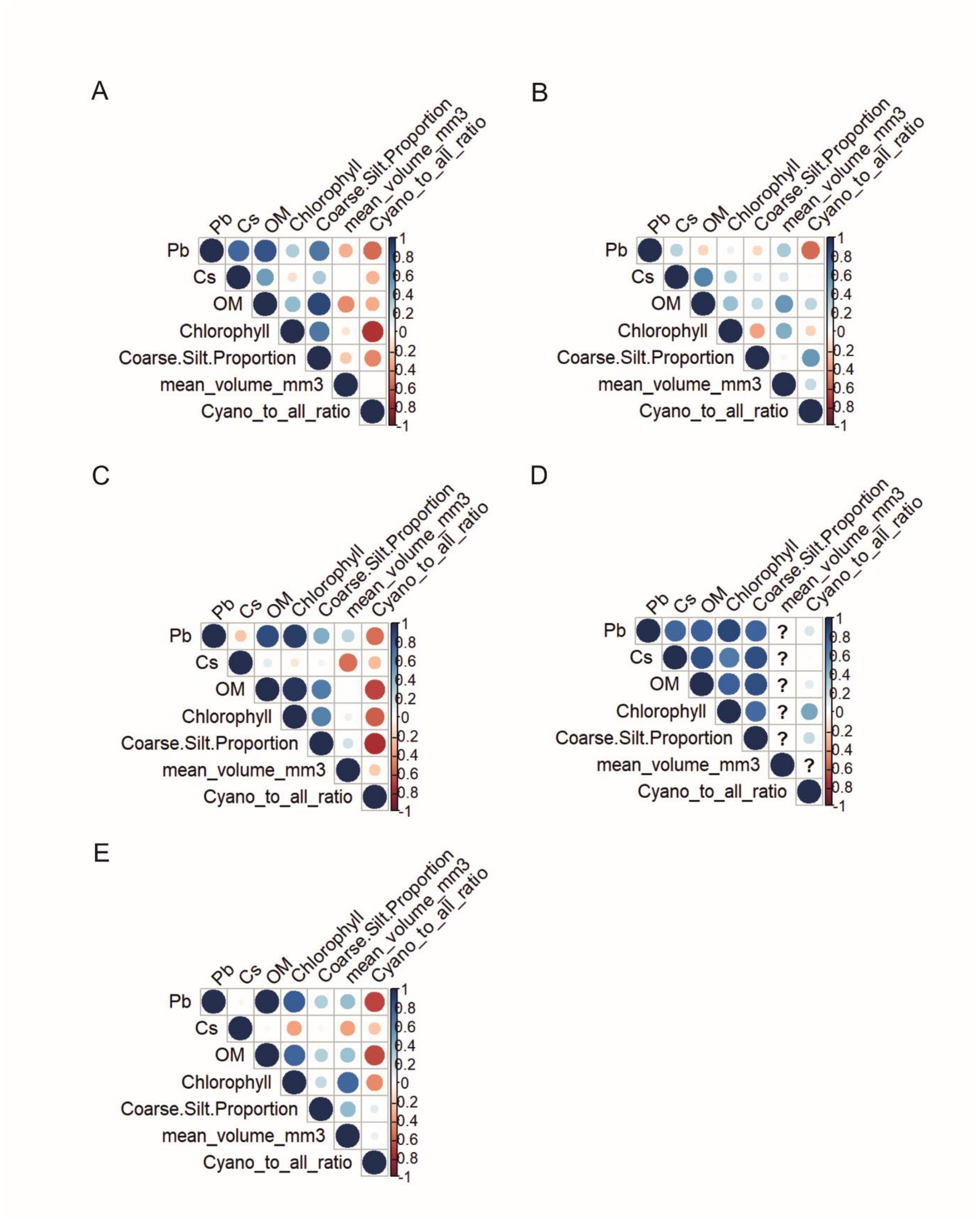


Figure S1. Correlation of raw environmental variable considered in the modelling. Glaciers: A – Blanc, B – Mont Miné, C – Pasterze, D – Preda Rossa, E – Ventina.

Table S1. List of investigated glaciers. Correlation# refers to analysing 137Cs and 210Pb activity concentrations with biotic and abiotic factors. *average sampling altitude. For Correlation# analysis, 10 separate samples were analysed per glacier; for Uptake it was one polled sample (the reason explained in Methods); Mobility was analysed based on 4 samples per glacier, that were polled after extraction.

Glacier	**Coordinates (Latitude, Longitude)**	**Altitude* (m a.s.l)**	**Aim**	**Year**
Mont Miné Glacier	46.029, 7.573	2470	Correlation#	2021
Blanc	44.946, 6.397	3000	Correlation#	2021
Preda Rossa	46.261, 9.739	2860	Correlation#	2021
Ventina	46.272, 9.780	2520	Correlation#	2021
Pasterze	47.087, 12.724	2200	Correlation#	2021
Oberaar	46.536, 8.225	2380	Uptake	2021
Mittelbergferner	46.919, 10.896	2680	Uptake	2021
Morterasch	46.415, 9.934	2200	Uptake	2021
Pers	46.407, 9.958	2700	Uptake	2021
Lang	46.458, 7.932	2500	Uptake	2021
de Moiry	46.088, 7.594	2650	Uptake	2021
de Piece	45.999, 7.471	2800	Uptake	2021
Forni	46.397, 10.588	2670	Uptake, Correlation#, Mobility	2021 & 2020
Mandrone	46.184, 10.559	2670	Mobility	2020
Ebenferner	46.517, 10.463	3030	Mobility	2020
Cedec	46.449, 10.592	2950	Mobility	2020
Dosdè	46.393, 10.216	2750	Mobility	2020

Table S2. Loss of reads through quality control of 16S rDNA V4 amplicon data.

Sample_ID	Glacier	Input	Filtered	Denoised	Nonchim	Run
121	Ventina	68629	31030	30160	26016	r1
122	Ventina	45353	19728	19313	17664	r1
122	Ventina	117961	3858	3672	3672	r2
123	Ventina	84181	37040	35944	28277	r1
124	Ventina	79649	34071	33420	28611	r1
125	Ventina	56708	24256	23612	20873	r1
126	Ventina	72156	34493	33354	28360	r1
127	Ventina	102607	44031	42805	34948	r1
128	Ventina	2106038	43663	42790	33527	r1
129	Ventina	356525	24	8	8	r1
130	Ventina	489717	35987	34728	28255	r1
130	Ventina	95672	3195	2913	2913	r2
131	Preda Rossa	551683	32853	31786	25268	r1
131	Preda Rossa	102688	3306	3111	3038	r2
132	Preda Rossa	1987029	37455	36485	32426	r1
132	Preda Rossa	160423	7019	6730	6299	r2
133	Preda Rossa	531573	13422	12734	11663	r1
133	Preda Rossa	143334	5949	5622	5580	r2
134	Preda Rossa	551865	21410	20487	18416	r1
134	Preda Rossa	204205	12955	12564	12377	r2
135	Preda Rossa	1063816	37982	36430	31547	r1
135	Preda Rossa	82062	4560	4268	4252	r2
136	Preda Rossa	616630	49511	48166	39134	r1
136	Preda Rossa	55341	1858	1750	1708	r2
137	Preda Rossa	725919	51230	49899	39990	r1
137	Preda Rossa	71320	2978	2765	2680	r2
138	Preda Rossa	524085	25181	24398	21436	r1
138	Preda Rossa	69698	2512	2347	2288	r2
139	Preda Rossa	470850	34722	33554	28394	r1
139	Preda Rossa	69046	3257	3088	3005	r2
140	Preda Rossa	476553	37855	36163	30942	r1
140	Preda Rossa	129846	8095	7728	7728	r2
141	Pasterze	1407673	40822	39931	35673	r1
141	Pasterze	125210	5387	5113	5113	r2
142	Pasterze	1724933	41575	40722	34609	r1
142	Pasterze	119855	8975	8762	8193	r2
143	Pasterze	823662	34509	33542	29216	r1
143	Pasterze	56466	3468	3319	3319	r2
144	Pasterze	474484	39942	38997	31835	r1
144	Pasterze	110820	7496	7234	6584	r2
145	Pasterze	60478	25519	23791	20286	r1
146	Pasterze	62011	25848	24222	21145	r1
147	Pasterze	67119	27558	26096	23469	r1
148	Pasterze	57620	24265	22925	19713	r1
149	Pasterze	67205	26396	25118	23277	r1
150	Pasterze	91731	39316	37652	32582	r1
151	Blanc	156722	77779	76055	61258	r1
152	Blanc	140438	68546	66959	46531	r1
153	Blanc	124669	60521	58915	44247	r1
154	Blanc	51030	23646	22772	18211	r1

155	Blanc	78277	35841	35039	26285	r1
156	Blanc	110458	47603	46308	34309	r1
157	Blanc	81621	35658	34621	28301	r1
158	Blanc	126429	45531	44350	33782	r1
160	Blanc	107144	4590	4309	4219	r2
161	Mont Miné Glacier	112529	8680	8456	8132	r2
162	Mont Miné Glacier	38815	2447	2335	2303	r2
163	Mont Miné Glacier	37959	3218	3007	2966	r2
165	Mont Miné Glacier	196048	15585	15032	12444	r2
166	Mont Miné Glacier	150648	13378	12813	11680	r2
167	Mont Miné Glacier	79729	6140	5806	5380	r2
168	Mont Miné Glacier	258313	119126	116125	89926	r1
169	Mont Miné Glacier	327405	177651	174720	141286	r1
170	Mont Miné Glacier	199705	102975	101100	85787	r1
K1isolation		291545	142040	140315	129721	r1
K1pcr		21338	10932	10878	10878	r1
K1pcr		11719	1355	1332	1332	r2
K2isolation		179059	91955	90285	81784	r1
K2pcr		246208	119665	118310	110118	r1
K3isolation		233227	125660	124392	117188	r1
K4isolation		261603	130606	128580	115593	r1

Table S3. Masses of samples used in radiometric measurements. For mobility analysis, masses were equal to the sediment mass it was extracted.

Sample type	Spectroscopy	Mean	Min	Max
Cryoconite	alpha	0.561 g	0.513 g	0.659 g
Cryoconite	gamma	1.192 g	0.287 g	4.361 g
Sediment under the stones	gamma	1.523 g	0.363 g	3.713 g
Animals	alpha	0.038 g	0.026 g	0.062 g
Animals	gamma	0.051 g	0.026 g	0.082 g

Table S4. Average activity ratio of ^{238}Pu$^{/239+240}$Pu in polled cryoconite samples.

Glacier	$^{238/239+240}$Pu	SD
Blanc	0.042	0.008
Ferpecle	0.070	0.028
Pasterze	0.033	0.018
Preda Rossa	0.031	0.008
Ventina	0.043	0.006

Table S5. Uptake ratio of radioisotopes in springtails compare to other habitats.

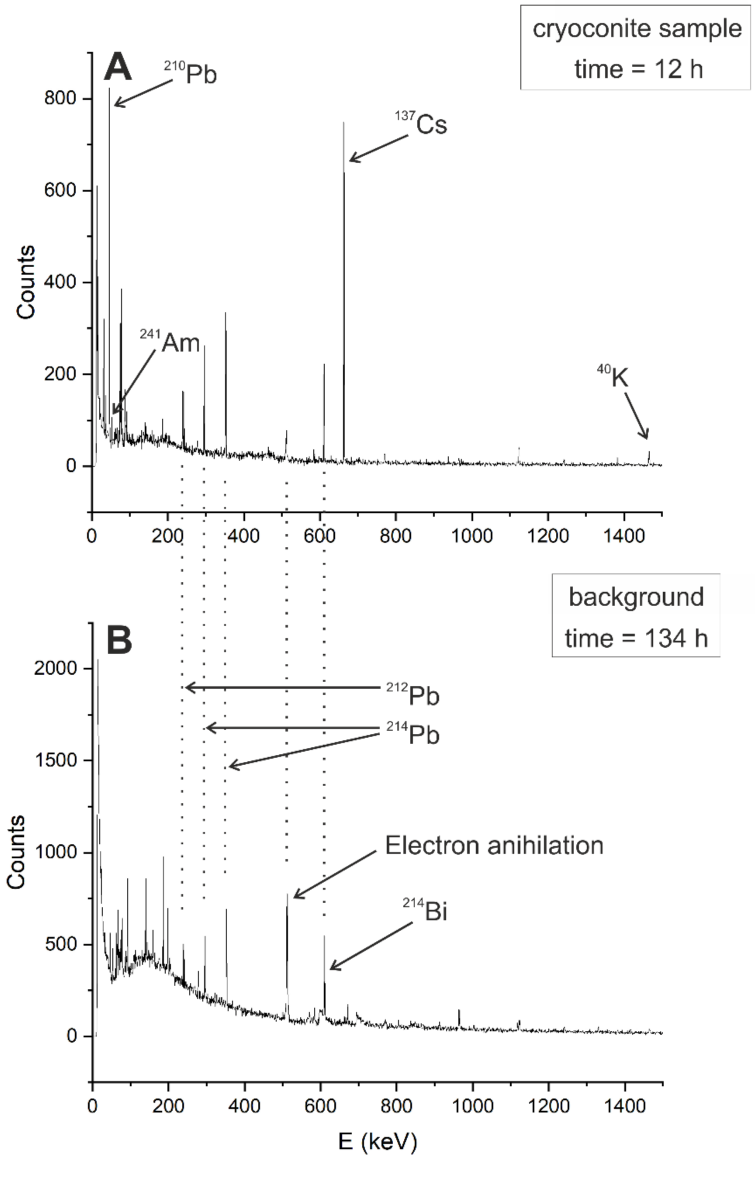
Animal	**Trophy group**	**Ecosystem**	**^{137}Cs**	**$^{239+240}$Pu**	**Reference**
Springtails	Detrivorous?	Glaciers	0.07(0.111)	0.07	This study
Insect (Ants)	Omnivorous	Forest	0.01-0.04	–	(Dragović & Mandić, 2010)
Malacostraca	Detrivorous	Forest	0.13-0.73	0.26-1.8	(Copplestone et al., 1999)
Oligochaeta	Detrivorous	Forest	0.9-1.33	–	(Copplestone et al., 1999)
Araneida	Predators	Forest	0.43-3.17	0.74-2.28	(Copplestone et al., 1999)
Carabidae	Predators	Forest	0.04-3.22	0.02-0.98	(Copplestone et al., 1999)
Opiliones	Predators	Forest	0.37-1.63	0.14-21.0	(Copplestone et al., 1999)

References

Copplestone, D., Johnson, M. S., Jones, S. R., Toal, M. E., & Jackson, D. (1999). Radionuclide behaviour and transport in a coniferous woodland ecosystem: vegetation, invertebrates and wood mice, Apodemus sylvaticus. *Science of The Total Environment*, *239*(1–3), 95–109. https://doi.org/10.1016/S0048-9697(99)00294-6

Dragović, S., & Mandić, L. J. (2010). Transfer of radionuclides to ants, mosses and lichens in semi-natural ecosystems. *Radiation and Environmental Biophysics*, *49*(4), 625–634. https://doi.org/10.1007/S00411-010-0319-8/TABLES/8

Supplementary materials – Chapter 2



Supplementary Figure 1. Gamma radiation spectrum. The upper panel shows the spectrum of one sample measured over 12 hours. Peaks of ^{210}Pb, ^{137}Cs, and ^{241}Am are marked, and a small peak of ^{40}K above 1400 keV is present. On the lower panel, the background spectrum is presented with marked peaks of ^{212}Pb, ^{214}Pb, and ^{214}Bi, whose presence in the sample spectrum is due to the background radiation.

Supplementary Table 1. Glacier-specific variables. *These variables were estimated based on the GLIMS database except Pers (Google Earth data).

Glacier	**Lat**	**Lon**	**Avg. altitude [m a.s.l.]***	**Max. altitude [m a.s.l.]***	**Size* [km^2]**	**Estimation_year**	**Prec_GF [mm]**	**Prec_CHB [mm]**
1. Cedech	46.449	10.598	3322	3720	2.04	2016	2379	75
2. Dosde	46.393	10.216	2861	3221	0.59	2016	1840	68.9
3. Morteratsch	46.416	9.933	3079	3985	14.59	2015	2090.6	89.3
4. Forno	46.323	9.7	2766	3289	4.95	2016	2422.6	95.2
5. Rhone	46.584	8.390	2969	3610	14.18	2015	2666.2	108.5
6. Aletsch	46.442	8.085	3073	4128	83.38	2015	2543.6	114.4
7. Ventina	46.276	9.780	2813	3532	1.71	2016	2257.2	93.3
8. Pers	46.408	9.959	2900	3903	6.40	2022	2354.2	82.6
9. Forni	46.397	10.588	3153	3663	10.50	2016	2245.4	71.7
10. Ebenferner	46.515	10.462	3109	3452	3.40	2016	1940.4	70.1
11. Lang	46.458	7.932	3278	3491	8.01	2015	1882.7	72
12. de Moiry	46.088	7.589	3226	3659	4.04	2015	2202	87
13. Oberaar	46.536	8.223	3186	3382	4.228	2015	2587.7	113.3
14. de Piéce	46.001	7.470	2975	3678	1.14	2015	3180.3	125.1
15. Mittelberg	46.919	10.896	3117	3390	8.06	2015	2959.6	113.9
16. Mont Miné	46.028	7.572	3203	3659	8.69	2015	2003.3	77.9
17. Blanc	44.946	6.397	3233	4054	4.70	2015	3315.9	96
18. Preda Rossa	46.260	9.739	3040	3443	0.48	2016	2295.8	93.9
19. Pasterze	47.087	12.724	2914	3495	16.17	2016	5063.3	154
MIN	-	-	2766	3221	0.48	-	1840	68.9
MAX	-	-	3322	4128	83.38	-	5063.3	125.1
MEAN	-	-	3064	3618	10.38	-	2538.4	94.8

Supplementary Table 2. Activity concentration of ^{210}Pb, ^{137}Cs, ^{241}Am, and organic matter content average per glacier based on 5-10 cryoconite samples collected in 2020 and 2021. BDL refers to values below the detection limit in all samples from a glacier. The ^{241}Am_h column represents the activity concentration of ^{241}Am with activity concentration as half of the minimum detectable concentration. ^{M} the average of minimal detectable activity concentration will be two times higher than the value in the ^{241}Am_h column.

Glacier	**^{210}Pb (Bq kg^{-1})**		**^{137}Cs (Bq kg^{-1})**		**^{241}Am (Bq kg^{-1})**		**^{241}Am_h (Bq kg^{-1})**		**OM [%]**	
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
1. Cedech	2115	624	1424	625	19.1	4.2	14.3	8.5	5.69	1.2
2. Dosde	10652	1844	4808	2205	42.4	26.8	42.4	26.8	12.78	1.64
3. Morteratsch	3325	813	581	380	18.9	-	7.3	6.8	7.10	1.41
4. Forno	3478	767	46	40	BDL^{M}	-	0.2	0.4	5.71	1.48
5. Rhone	6121	502	326	129	BDL^{M}	-	0.3	0.7	11.91	0.84
6. Aletsch	3593	822	402	333	BDL^{M}	-	0.1	0.2	10.49	2.47
7. Ventina	7237	3146	5785	2804	36.6	19.3	33.3	21.1	10.21	3.09
8. Pers	4965	690	3399	1653	39.8	6.9	25.7	20	7.37	1.09
9. Forni	5420	709	185	233	BDL^{M}	-	2.5	0.9	10.24	1.29
10. Ebenferner	10968	1011	1700	390	BDL^{M}	-	4.8	4.2	14.01	1.59
11. Lang	661	318	310	166	12.0	-	3.7	4.8	1.70	0.41
12. de Moiry	3028	413	1703	983	40.2	7.2	25.0	21.5	8.36	1.31
13. Oberaar	2365	1487	36	35	BDL^{M}	-	0.6	0.8	4.45	2.04
14. de Piéce	6381	919	3076	2072	27.6	9.1	17.9	14.7	9.07	1.5
15. Mittelberg	4530	1192	1294	211	BDL^{M}	-	0.7	1	9.97	1.71
16. Mont Miné	5950	2210	3820	1430	17.8	7.8	7.3	6.9	16.50	1.03
17. Blanc	3845	1448	2982	1567	26.0	14.3	23.9	15	5.82	1.53
18. Preda Rossa	2270	1099	802	584	20.3	3.5	4.9	8.2	3.49	1.06
19. Pasterze	1556	675	239	83	BDL^{M}	-	0.4	0.5	3.53	1.2
MIN	*661*	-	*36.3*	-	*11.9*	-	*0.1*	-	1.70	-
MAX	*10968*	-	*5785*	-	*42.4*	-	*42.4*	-	16.50	-
MEAN	*4656*	-	*1733*	-	27.3	-	11.3	-	8.34	-

Supplementary Table 3. The results of quality assurance for reference material CRM IAEA-447 (INTERNATIONAL ATOMIC ENERGY AGENCY, Certified Reference Material Certificate IAEA-447, IAEA, Vienna, 1-7 pp.)

Reference material/standard (reference date)	**^{210}Pb (Bq kg^{-1})**		**^{241}Am (Bq kg^{-1})**		**^{137}Cs (Bq kg^{-1})**	
	measured	*certified*	*measured*	*certified*	*measured*	*certified*
IAEA 447 (2021-03-01)	324 ± 4 (n=3)	306 ± 15	2.2 ± 0.2 (n=3)	2.3 ± 0.2	306 ± 13 (n=3)	328 ± 8

Supplementary Table 4. Comparison of models based on reduction of Residuals Sum of Squares (RSS). The best models are bolded that explained the greatest proportion of variation and contains no outliers based on Cook's distance.

^{210}Pb

Model 1: log(^{210}Pb) ~ 1
Model 2: log(^{210}Pb) ~ OM
Model 3: log(^{210}Pb) ~ OM + Average_altitude
Model 4: log(^{210}Pb) ~ OM + Size_kmq_l
Model 5: log(^{210}Pb) ~ OM + Size_kmq_l + Average_altitude

Model	Res. df	RSS	Delta df	Delta RSS	F	P	No. of detected outliers
1	18	8.2935					
2	17	2.3877	1	5.9059	74.3878	< 0.001	2
3	16	1.6045	1	0.7831	9.8639	0.0067	1
4	16	1.7732	0	-0.1686			1
5	**15**	**1.1909**	**1**	**0.5823**	**7.3340**	**0.0162**	**0**

^{137}Cs

Model 1: log(Cs137) ~ 1
Model 2: log(Cs137) ~ OM
Model 3: log(Cs137) ~ OM + Size_kmq_l
Model 4: log(Cs137) ~ OM + prec_GLF_l + prec_CHB_l
Model 5: log(Cs137) ~ OM + Size_kmq_l + prec_GLF_l + prec_CHB_l

Model	Res. df	RSS	Delta df	Delta RSS	F	P	No. of detected outliers
1	18	40.483					
2	17	31.823	1	8.6604	5.4663	0.03475	0
3	**16**	**24.388**	**1**	**7.4347**	**4.6927**	**0.04804**	**0**
4	15	28.540	1	-4.1519			0
5	14	22.180	1	6.3595	4.0140	0.06487	0

Author's contribution statement

AUTHORSHIP CONTRIBUTION STATEMENT

I am the lead and corresponding author in the following articles included in my doctoral dissertation. I conceptualized both articles under the supervision of prof. Krzysztof Zawierucha and prof. Edyta Łokas. Detailed contributions are listed below.

Article

Buda, J., Łokas, E., Błażej, S., Gorzkiewicz, K., Buda, K., Ambrosini, R., Franzetti, A., Pittino, F., Crosta, A., Klimaszyk, P., Zawierucha, K. (2024). Unveiling threats to glacier biota: Bioaccumulation, mobility, and interactions of radioisotopes with key biological components. Chemosphere, 348, 140738.

Contribution:

Conceptualization, Methodology, Resources, Sampling, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Funding acquisition.

Article

Buda, J., Błażej, S., Ambrosini, R., Scotti, R., Pittino, F., Sala, D., P., Zawierucha, K., Łokas, E. The surface of small glaciers as radioactive hotspots: concentration of radioisotopes during predicted intensive melting in the Alps. (under review)

Contribution:

Conceptualization, Methodology, Resources, Sampling, Investigation, Formal analysis, Data curation, Visualization, Writing - original draft, Funding acquisition.

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