PATHOGENS IN PERMAFROST: THE NEXT PANDEMIC?

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Insight

'Russia anthrax outbreak affects dozens in north Siberia' (BBC news) [1]; 'The permafrost pandemic: could the melting Arctic release a deadly disease?' (Greenpeace) [3]; 'Deep Frozen Arctic Microbes Are Waking Up' (Scientific American) [4]; 'All hell breaks loose as the tundra thaws' (The Guardian) [5]; 'How thawing permafrost could resurrect long-dormant diseases' (Arctic Today) [6].

These are just some of the headlines published in the last few years regarding thawing permafrost and the potentially pathogenic microorganisms it contains. While these scenarios might once have seemed like the plot of a semi-interesting apocalypse movie, the COVID-19 pandemic and the unusually large 2021 wildfires raging across the Arctic circle have suddenly made worrying about thawing permafrost releasing some unknown ancient disease much more rational. But, can these diseases still form a threat to us? And if so, how is this possible? This editorial will give a brief overview of the current state of microorganisms in permafrost and their potential to cause the next big outbreak.

For the third summer in a row, the coldest region of Russia was on fire. In Yakutia, in the Northeast of Siberia, one of the hottest and driest summers was recorded in over a 100 years with temperatures up to 38 °C. According to the Guardian, the wildfires destroyed a record-breaking 18.16 million hectares, which is over four times the size of the Netherlands. For the first time in recorded history, the smoke reached the north pole 3000 kilometres away, darkening the snow and ice and causing it to melt faster due to increased heat absorption from the sun [7, 8].

It is not unusual to have some summer fires in this region, and they actually support the ecosystem's health, but the summer of 2021 has proven to be exceptional [9, 10]. In addition to the fires releasing about 970 megatons of carbon dioxide into the atmosphere - twice as much as was released in the previous year - the relentless heat also accelerates thawing of the Siberian permafrost, partly due to increased microbial activity [9, 11]. Besides storing enormous amounts of methane and carbon, which contribute to global warming upon release, permafrost also contains a whole zoo's worth of frozen microorganisms [12]. This raises the question: should we be worried about the microorganisms preserved in these frozen areas causing the next pandemic?

Permafrost

Before we get to this, let us dive into what permafrost exactly is. Classically, permafrost is defined as soil that has remained frozen for at least two years in a row, but it can be millions of years old [11]. About 25% of the land on Earth is underlain by permafrost [11]. Overlaying this permafrost is the so-called 'active layer', which is subject to seasonal freeze-thaw cycles. Therefore, melting of a few centimetres up to several meters of permafrost is normal. However, due to global warming, the depth of the active layer has been increasing, resulting in a decrease of the permanent permafrost [13]. This is where thawing permafrost becomes problematic. Permafrost has accumulated organic matter, such as remnants from plants and animals, over many, many years. When temperatures rise, microorganisms that were once frozen can be reactivated and start decomposing these remnants (figure 1). As a result of this, carbon dioxide (CO_2) and methane (CH_4) are released; these gasses could possibly accelerate global warming and, therefore, even more thawing of permafrost [11, 14]. This is already happening: while in the last 100 years, the global average temperature increased by 0.7 °C, the average temperatures of the upper layer of Arctic permafrost increased by 3.0 °C, a phenomenon known as 'Arctic Amplification' [15]. According to one projection, this could lead to a 90% reduction of near-surface permafrost by 2100 [16]. Theoretically, thawing of the permafrost could then also release reawakened historical pathogens that might pose a threat for future outbreaks [17, 18].

Surviving the cold

It seems quite impossible for any organism to remain viable after being frozen for hundreds to thousands of years. However, microorganisms have developed several strategies that enable them to survive in temperatures below 0 °C. One of these is to enter a dormant state, where there is low metabolic activity and production of specialised proteins that help the cell survive [19]. In 2007, researchers found bacteria that survived frozen conditions for 25,000 years [20]. Another recent example is a study where an Acanthamoeba was used as 'bait' to isolate a large, viable DNA virus from a 30,000-year-old permafrost layer [21]. The microorganisms do not just survive but are shown to be metabolically active and grow at temperatures up to -20 °C [22]. Even when investigating the oldest ice layers on Earth of approximately 34-million-years old, viable metabolically active bacteria were found that had about eight million birthdays to their name [23].

And it is not just all geriatric pathogens chilling out in the guts of long-dead mammoths [24]. We, as humans, are also regularly depositing new microorganisms on the ice. One study performed on the Kahiltna Glacier in Alaska, a popular climbing spot, calculated that climbers annually produced over two megatons of human waste [25]. Besides the faeces leading to highly unhygienic situations that resulted in diarrhoea for almost a third of all 132 interviewed climbers, the bacteria in the faeces also turned out to be able to survive for an

Permafrost can not only be found on our planet, but also on Mars, Jupiter, Saturn, Uranium, Neptune and Pluto. Intriguingly, if microorganisms are surviving in the Earth's permafrost, there might also be cellular life on the coldest planets of our Solar System [2].

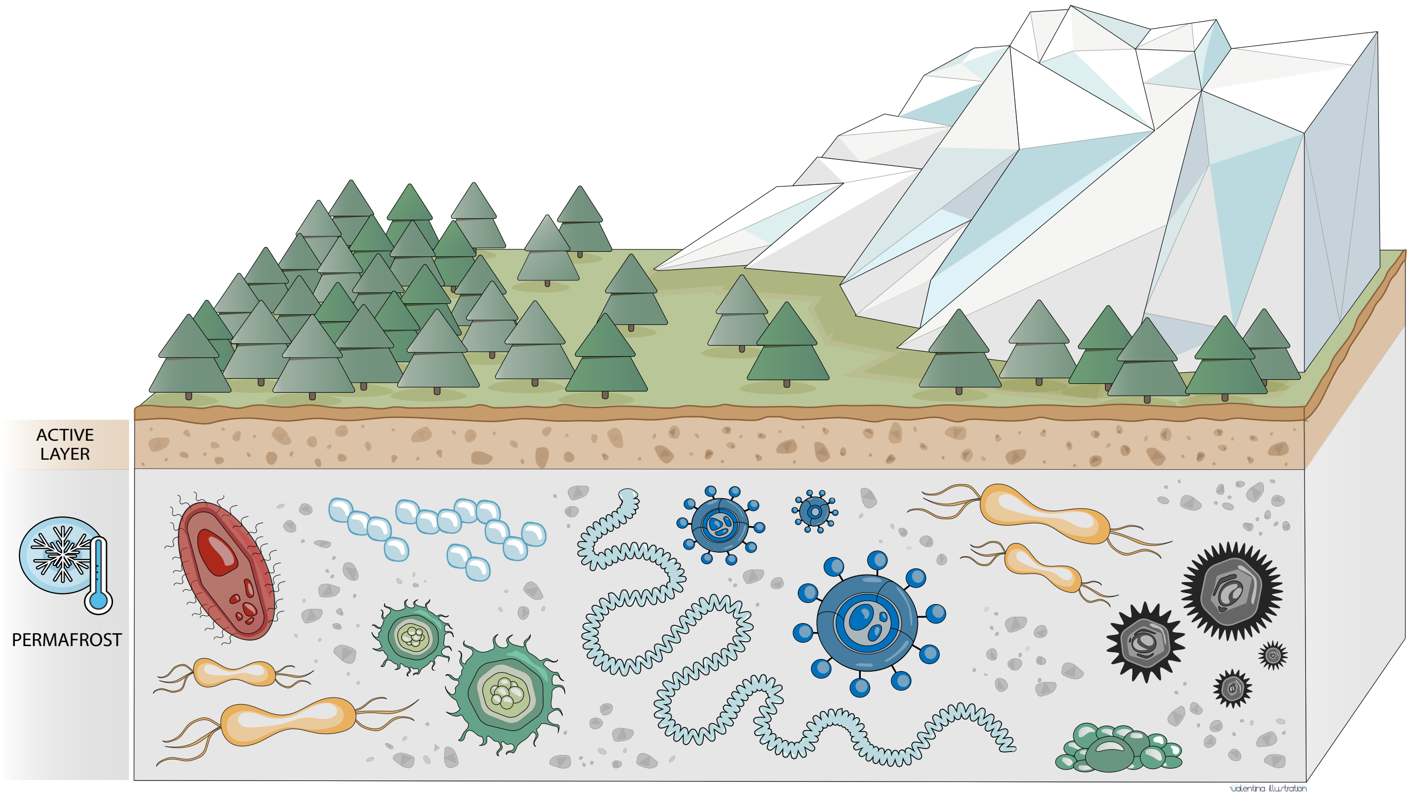

***Figure 1:** A simplified image of the active layer and the micro-organisms in permafrost (not to scale).*

extended period of time and were predicted to travel through the glacier resurfacing at lower, more accessible, elevations [25]. Thus, both old and new microorganisms can be found in permafrost.

A real threat?

In practice, dangerous, living pathogens caused a large anthrax outbreak in 2016 in Arctic Russian Siberia [18]. This resulted not only in the untimely demise of over 2000 reindeer but also in the hospitalisation of 20 people and eventually the tragic death of a 12-year old child [18]. The victims were infected by spores of *Bacillus anthracis* that had been preserved in frozen soil for over 75 years [18]. Experts believe that spores were released from previously frozen burial grounds of infected carcasses that surfaced due to high thawing rates [26]. As there are up to 13,885 cattle burial grounds in Russia alone, primarily because of anthrax outbreaks, re-emerging anthrax provides a real threat to those living nearby [27].

Following this concept, similar outbreaks could occur due to other robust pathogens. One of the more infamous candidates may be the variola virus, which causes smallpox. Before this devastating disease was eradicated in 1980, it is estimated to have been the cause of 10% of all deaths worldwide during the last millennium [28]. Smallpox vaccination campaigns were abolished decades ago, increasing the fraction of the world's population that has no immunity against smallpox. Combine this with the rise in immunosuppressed individuals, and smallpox suddenly reappears as a significant threat [29].

Already in 1991, when a grave filled with 19th century mummified smallpox victims were found, the Russian authorities were worried that floods might wash the virus to inhabited regions [30]. A similar burial ground was unearthed in 2004 when French and Russian researchers found a wooden grave in the permafrost containing five mummies [31]. Samples from this uncanny discovery revealed that the most likely cause of death was variola infection. The researchers could link this particular strain to the smallpox epidemic over 300 years earlier in 1714 [31].

Fortunately, until now, no viable virus has ever been isolated from a mummified smallpox victim. However, researchers do not entirely exclude the possibility that there may be viable variola viruses buried somewhere in the Arctic [32]. It is a Schrödinger's cat situation: as long as we cannot find it, it might be alive, and it might be dead.

Additional danger can be found in the potential for permafrost to act as a reservoir for antibiotic resistance genes. Using PCR techniques, researchers have found resistance genes for antibiotics such as beta-lactams and tetracycline, that possibly spread to these areas by migrating birds and airborne bacteria [33, 34]. In a world where antibiotic resistance is one of the top ten global health problems, such a reservoir periodically releasing resistance genes may become problematic [35].

This 'will they, won't they' seems to be a recurring trope when researching this topic; there might be a viable and devastating disease hiding in this Pandora's box of microorganisms, but there might also be none. Some researchers argue that the freeze-thaw cycles necessary to incorporate microorganisms into the permafrost as well as the subsequent thaw and release are likely to inactivate microorganisms [36, 37]. In addition, permafrost soil is generally acidic, limiting survival [34, 37]. Even the previously described Siberian anthrax outbreak – often invoked to emphasize the dangers of frozen pathogens – is now questioned to be solely caused by permafrost thawing; the discontinued reindeer vaccinations and increased reindeer numbers are very likely to have contributed as well [37].

Resurrecting viruses

There may be more danger in scientists resurrecting viruses from the viral genomic material that is preserved. For example, researchers previously managed to recover the entire viral genome of the 1918 Spanish flu, which was responsible for over 50 million deaths [38]. While this does provide essential information on, for example, the prevention and control of future pandemics - which is relevant for obvious reasons - certain dangers are attached. In 2014, researchers recovered the viral genome of two unknown viruses from 700-year old caribou faeces, one of which was able to infect plants under laboratory conditions [39]. Since there is not much known about the infectivity of these viruses, this kind of research must be done with great care for safety if we want to avoid 'pathogens escaping from the lab' scenarios [34].

Conclusion

So, in the context of thawing permafrost, pandemics are not likely to be one of the major problems. There are plenty of other issues to worry about regarding warming and thawing of permafrost soil, such as increased intensity and frequency of wildfires; infrastructure collapse due to damage to roads, buildings, pipelines and houses; coastal erosion and flooding; poleward disease-vector spread of, for example, ticks; and let us also not forget permafrost stores up to 1580 billion megatons of organic carbon, almost twice as much as is present in the entire atmosphere [14, 36, 37]. Gradual emissions of these thawing stocks into the atmosphere will likely have significant effects on global warming.

However, it never hurts to be safe on the health side. We simply do not know the extent of the danger yet, and more research is necessary to determine what microorganisms in permafrost are actually viable and able to cause an outbreak upon release. Especially since climate change, 'last chance'-tourism, and increased oil, gas, and mineral extraction in the Arctic circle lead to higher levels of direct human contact with thawing permafrost, monitoring and surveillance of the permafrost and its microorganisms might not be a luxury [40, 41]. We are already dealing with one pandemic. Let us not make it more!

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CORRECT ANSWERS TO THE EXAM QUESTIONS

Answer question 1:
B. Hyperpolarisation

Chloride ions are negatively charged. When these negative ions flow into the cell, the membrane potential will become even more negative than the resting membrane potential as more negative charge will be inside the cell compared to outside the cell. When this happens, we talk about hyperpolarisation of the membrane potential. The hyperpolarisation will cause an inhibitory effect as more positive charge is needed to depolarise the cell. Glycine is one of the major inhibitory neurotransmitters.

For further reading:
Siegel, A., Sapru, H. Chapter 7: Neurotransmitters in Essential Neuroscience, 4th edition (Wolters Kluwer, Philadelphia, 2019).
Siegel, A., Sapru, H. Chapter 6: Synaptic Transmission in Essential Neuroscience, 4th edition (Wolters Kluwer, Philadelphia, 2019).

During the exam, 44% of the participants answered this question correctly.

The exam questions can be found back on page 24 in this journal.

Answer question 2:
C. tumour cells have also been found in the lymph nodes, but no distant metastases have yet been detected.

Colon carcinomas can be staged by the use of the TNM-classification. T3 stands for tumour invasion into the subserosa, N2 shows that there are metastases in four or more regional lymph nodes, and M0 indicates that there are no distant metastases. Therefore, tumour cells have been found in the lymph nodes, but not in other distant organs.

For further reading:
Tanis P. J., Beets-Tan R. G. H., Marijnen C. A. M., Nagtegaal I. D., Punt C. J. A. Chapter 10: Tumoren van dunne en dikke darm in Leerboek oncologie, 9th edition (Bohn Stafleu van Loghum, Houten, 2017)

Akkoca, A. N., Yanık, S., Ozdemir, Z. T., Cihan, F. G., Sayar, S., Cincin, T. G., Cam, A., & Ozer, C. (2014). TNM and Modified Dukes staging along with the demographic characteristics of patients with colorectal carcinoma. *International journal of clinical and experimental medicine*, 7(9), 2828–2835.

During the exam, 96% of the participants answered this question correctly.