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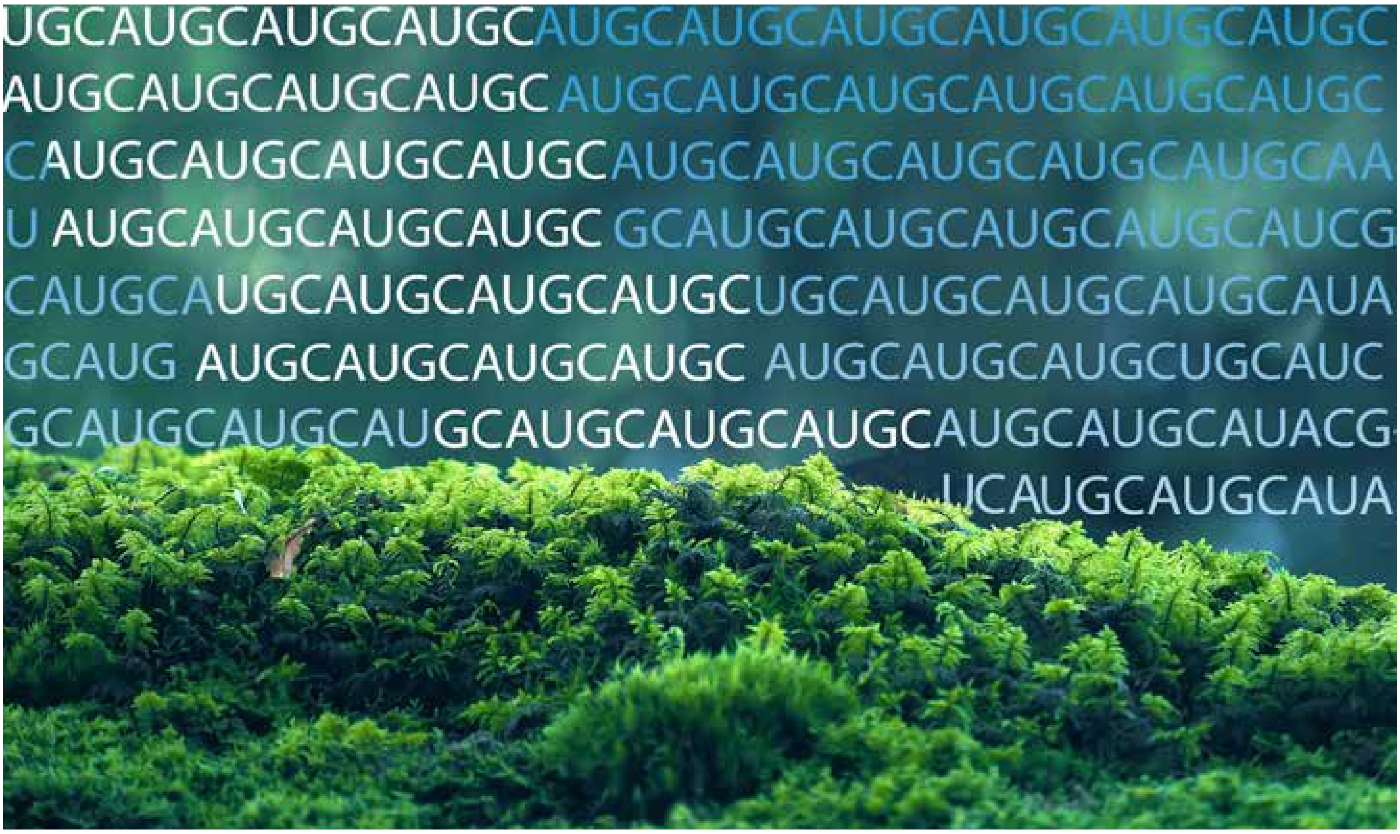
Purifying mining wastewater with plant-associated microbes

Kaisa Lehosmaa studies the potential of Arctic microbes and plants to purify industrial wastewater. Such method can help to reduce environmental contamination caused by the mining industry. In addition to mining, nitrogen and heavy metal loads to waterbodies come from wastewater treatment plants, stormwater, agriculture, and peat production. The suitable plant-associated microbes for bioremediation are mainly determined by sequencing methods, but also by isolating microbes from the moss.



Industrial processes such as mining have led to increased concentrations of nitrogen and heavy metals in soil and water. Post-doctoral researcher Lehosmaa works in **Anna Maria Pirttilä's** research group at the University of Oulu, studying endophytic microbes living inside moss and other plants and their suitability for water purification. The use of microbial 'symbionts' in bioremediation is a relatively little researched field. Some plants can store or even evaporate harmful substances such as metals and nutrients into the atmosphere. Plant-associated microbes play an important role in nutrient and metal uptake and transformation within the plants.

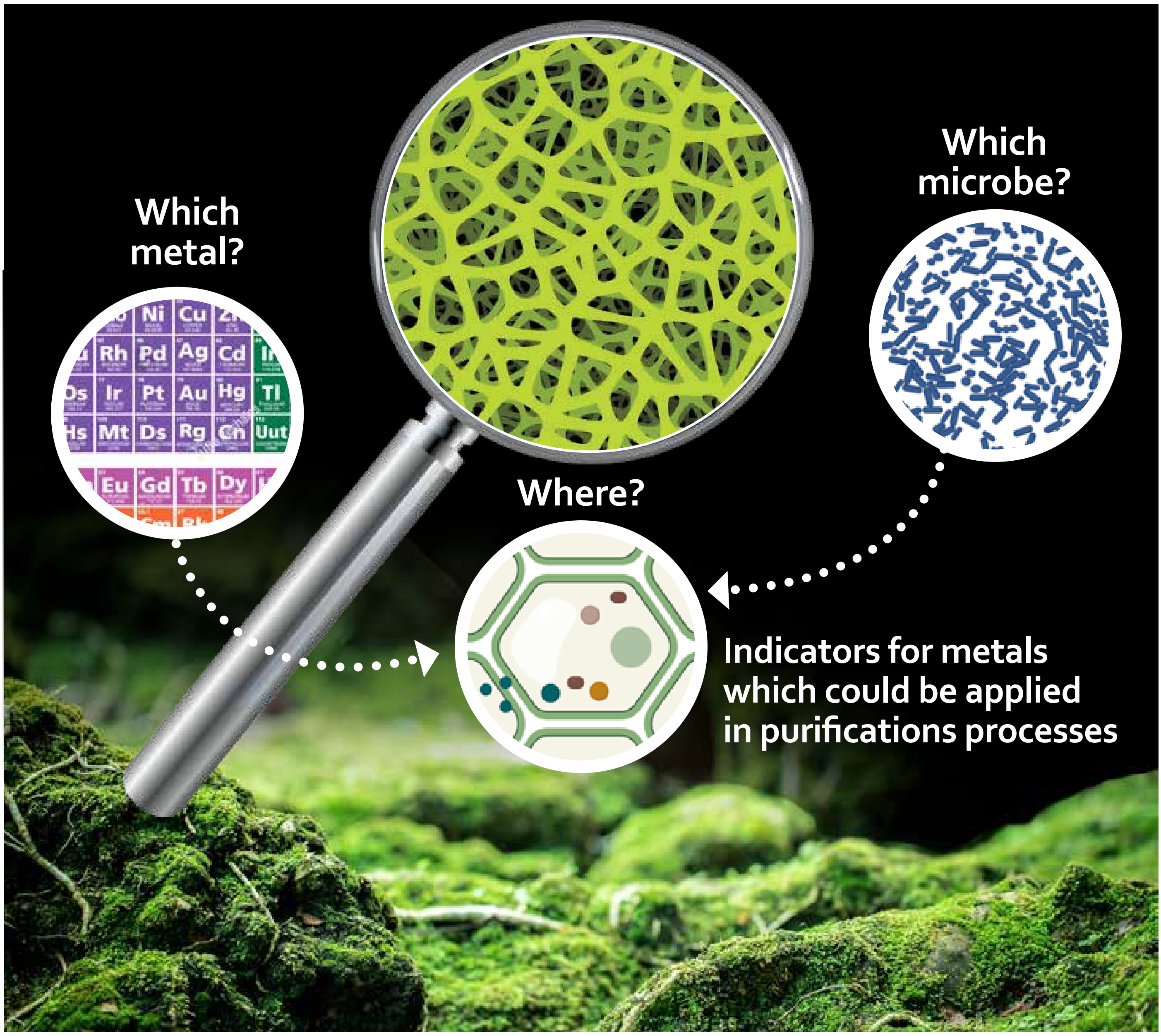
Floating hook-moss, a plant with high potential

In particular, Lehosmaa has studied floating hook-moss, *Warnstorfia fluitans*, which grows in Finland in low-nutrient peatlands and in groundwater-dependent spring ecosystems. The moss also grows in Pyhäjärvi in areas around the Pyhäsalmi mine. Pyhäsalmi is Europe's deepest base metal mine, yielding copper and zinc.

"We have found this moss in a mining area, which is naturally adapted to the harsh conditions. The microbes found in floating hook-moss tissue can be used with the moss to improve purification of mining wastewater in cold climate conditions," Lehosmaa explains.

Mining activities create acidic and metal-rich wastewater that is made mobile by gravity. The wastewater is highly acidic, and contains high levels of metals considered harmful – zinc, aluminium, copper and cadmium. Such wastewater must be treated and cleaned carefully, as it is harmful to the environment.

In Lehosmaa's research, floating hook-moss proved to be an effective accumulator for metal-rich waters even at low temperatures. When combined with a woodchip bioreactor, the combined unit removed nitrogen particularly well. Lehosmaa and her colleagues used sequencing methods to identify the microbial symbionts of floating hook-moss.



The metals and microbes accumulated in the moss tissue are identified through sequencing and traditional microbiological cultivation methods. After identification, metals and microbes are localized in the moss tissue. Identification and localization are used to determine microbes adapted to metal-rich conditions, which could potentially be applied in purification processes. Purification processes are enhanced by adding microbes to the moss tissue.

"Sequencing gives an overall picture of the microbial diversity of the moss – that is, how many and what kind of microbes are present.

We also want to know which microbial genes are active under various conditions, so that we can understand how microbes could be used more widely in bioremediation."

Plant-associated microbes are poorly understood

To analyse microbiome composition, Lehosmaa has used the computational resources of the Finnish ELIXIR node's CSC – IT Center for Science and its Chipster software.

"The microbiome of the moss is quite unknown – the microbial symbionts of plants in general are relatively poorly known. We have identified symbionts in the moss using amplicon and genome sequencing."

Amplicon sequencing targets the specific gene regions, in this case, the 16S and ITS ribosomal RNA (rRNA) gene regions. The 16S and ITS rRNA gene regions have remained the same over millions of years in evolution

symbionts to change the solubility of metals. Bioremediation often uses live microbes to precipitate metals into particulate form, thereby making them easier to control and remove."

It is crucial that the microbes found can also be grown in the laboratory.

"We can't use microbes in purification purposes if we can't grow and thus add them to the same or different plant species to promote metal uptake processes," Lehosmaa says. Microbial symbionts help plants to survive in difficult biological conditions.

In addition to moss, one effective plant used in bioremediation is the common reed. Like moss, it absorbs harmful substances effectively. The common reed creates a large amount of biomass and grows easily. The algae in its rhizome binds soil structures and prevents blue-green algal blooms. The common reed is also used for metal recovery; called phytomining or agromining. Lehosmaa and her colleagues have mapped the accumulation of copper and zinc in the common reed at the Pyhäsalmi mine.

for bacteria and fungi, which is why these regions can be used to identify different species. The 16S and ITS rRNA gene regions are sequenced and identified through publicly accessible databases.

"After identifying the microbes, the next step is to find out what they do. We already have preliminary results that interesting processes take place within the moss tissue."

According to Lehosmaa, it is important to know what happens inside the moss and how the microbes are able to process metals.

"Acidic water usually contains metals in soluble form. It is not possible to remove metals from water, since they are inorganic compounds. However, we can use microbial

The next goal is to assess the ability of other natural plants, fungi, and bacteria adapted to northern climate conditions to remove nitrogen and metals from water.

"Since the indicator microbes can be cultivated and added, the next step is to expand the research to other mosses. It is interesting to find out whether the microbes can function as well in other plants than floating hook-moss."

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