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Research article

The uptake of Ni^{2+} and Ag^{+} by bacterial strains isolated from a boreal nutrient-poor bog

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Abstract: We studied the uptake of Ni^{2+} and Ag^{+} by bacterial strains of *Paenibacillus*, *Pseudomonas*, *Burkholderia* and *Rhodococcus* isolated from an acidic nutrient-poor boreal bog. The tests were run in two different growth media at two temperatures; +4 ℃ and +20 ℃. All bacterial strains removed Ni^{2+} and Ag^{+} from the solution with highest efficiencies shown by one of the *Pseudomonas* sp. and one of the *Paenibacillus* sp. strains. Highest Ni^{2+} uptake was found in 1% Tryptone solution, whereas the highest removal of Ag^{+} was obtained using 1% Yeast extract. Temperature affected the uptake of Ni^{2+} and Ag^{+}, but statistically significant difference was found only for Ni^{2+}. Based on tests carried out for the bacteria in nutrient broths and for fresh samples taken from varying depth up to seven meters from the ombrotrophic bog, from which the bacteria were isolated, we estimated that in *in situ* conditions of the bog the uptake of Ni^{2+} by bacteria accounts for approximately 0.02% of the total sorption in the uppermost moss layer, 0.01% in the peat layer, 0.02% in the gyttja layer and 0.1% in the bottom clay layer of the bog. For Ag^{+} the corresponding values were 2.3% in the moss layer, 0.04% in the peat layer, 0.2% in the gyttja and 0.03% in the clay layer.

Keywords: Ni; Ag; bacteria; boreal; bog; uptake; *Pseudomonas*; *Burkholderia*; *Rhodococcus*; *Paenibacillus*

1. Introduction

Pollution of the environment by harmful contaminants such as heavy metals and radioactive material results from anthropogenic, mainly industrial, activities. Heavy metals present a severe

threat to biota, because of their accumulation in water bodies, high toxicity and low biodegradability [1]. The toxic effects of heavy metals on soil microorganism activity is well known, and heavy metals are known to inhibit bacterial cell growth and to affect both cell division and cell viability [2,3]. On the other hand, long-lived radioactive nuclides originating from nuclear industry can pose harmful radiation risks to humans and other biota.

Nickel (Ni) mining and industrial manufacturing of stainless steel, batteries and accumulators, as well as Ni-electroplating and pigment and ceramics industry give rise to wastewaters containing undesired amounts of Ni [4–9]. Ni is released into the atmosphere from the combustion of coal, diesel oil and fuel oil, from the burning of waste and sewage as well as from other miscellaneous sources, like tobacco smoking [10–12]. From the radioecological point of view, ^{63}Ni (half-life 96 years) is an important nuclide in the nuclear power point and decommissioning wastes and ^{59}Ni is classified as a high priority radionuclide in the biosphere safety assessments of the disposal of spent nuclear fuel [13]. This is due to its long half-life of 76 000 years and dominance in the calculated possible overall biosphere radiation doses, resulting from hypothetical escape of spent nuclear fuel from the deep geosphere repository [13–15].

Ni belongs to the essential metals and it acts as an important component in many enzymes, which participate in a number of important metabolic reactions [1,9]. These metabolic reactions include ureolysis, hydrogen metabolism, methane biogenesis and acidogenesis [1]. However, Ni intake succeeding tolerable levels causes many types of disease including pulmonary fibrosis, renal edema, skin dermatitis and gastrointestinal distress [9]. In addition, Ni has been suspected to be an embryotoxin and teratogen [16].

Ni can occur in several different oxidation states, but under environmental conditions Ni(II) is prevalent [12]. In soils Ni exists in several forms including inorganic crystalline minerals or precipitates [17]. It also occurs complexed or adsorbed on different organic or inorganic cation exchange surfaces, or as a water-soluble free-ion or chelated to metal complexes in soil solution where a decrease in soil pH increases its mobility [17,18].

Silver (Ag) has been released to the environment through various industrial applications, such as photographic and imaging industry [19]. Ag is a non-essential metal and it can be highly toxic to a number of organisms, even at very low trace concentrations [20]. The Ag(I) ion also acts as an effective bactericide [21]. Ag compounds accumulate through food chains and they cause several diseases and disorders including corrosive damage of the gastrointestinal tract, diarrhea, respiratory irritation, discoloration of skin, vomiting, shock, convulsions and even death [22,23].

Ag(I) forms moderately insoluble compounds with sulphate (SO_4^{2-}) and sulphide, as well as with halides (Cl^-, Br^-, I^-) [20]. Soluble multihalide complexes of Ag are also possible [20]. Soil organic matter (SOM) is known to bind Ag and both humic and fulvic acids have been shown to have strong sorption capacities for Ag [20,24,25]. It has been estimated, that 5% of the total Ag found in soils is biologically available, but in contaminated soils, however, this may be enough to adversely affect the soil's micro- and macrobiological populations [20]. In spent nuclear fuel, ^{108m}Ag is contained in the Ag-In-Cd alloy of the control rods. In the biosphere safety assessment calculations ^{108m}Ag is assumed to be part of the radionuclides that are instantly released if a copper canister containing the spent fuel is penetrated by water (so called instant release fraction, IRF) and therefore it can cause a significant portion of the radiation dose caused by the radionuclides potentially released into the biosphere from the spent nuclear fuel repository [26].

Two types of accumulation processes of metals can be found in microorganisms, namely

bioaccumulation and biosorption. Bioaccumulation is an active transport mechanism in which energy is required and therefore it is dependent on the metabolic activity of the cell, which in turn can be significantly affected by the presence of stable and radioactive ions [27]. Bioaccumulation is typically a relatively slow process and requires time for uptake by the microorganism [28]. In contrast, biosorption, which can also be reversible, is relatively fast [27]. Biosorption comprises physicochemical interactions between the functional groups (hydroxyl, carboxyl, sulfhydryl groups and phosphate groups of lipids, proteins and polysaccharides) of the cell surface and the adsorbing metal and involves physical adsorption, ion exchange, complexation and precipitation [29,30]. External factors such as pH, organic material (complexing agents), other ions in solution, cell metabolic products (which may cause metal precipitation) and temperature affect biosorption processes [30]. Among microorganisms, it is mainly the bacterial cell wall that contains chemical compounds with sites capable of passive binding of metals. In addition, bacteria are excellent biosorbents because of their high surface-to-volume ratio and a high content of potentially active chemisorption sites, such as teichoic acid found in the cell walls of Gram-positive bacteria [32–34].

In recent years, research has been focused on the use of microorganisms in the removal and possible recovery of heavy metals and radionuclides from various industrial wastes [1,7,30,32,35]. These applications typically rely on biosorption, in which the actual active biomass consists of dead and metabolically inactive cells of algae, fungi or bacteria [36–41]. However, microorganisms potentially affect the behaviour of metals and radionuclides also under environmental conditions using active mechanisms [42–44].

In the boreal region, nutrient-poor bogs represent unique ecological niches, with distinct microbial populations. However, so far there is only limited knowledge about the metabolism of the microbes inhabiting these northern areas. In the present study we used strains of *Pseudomonas* sp., *Burkholderia* sp., *Paenibacillus* sp. and *Rhodococcus* sp. previously isolated from the boreal ombrotrophic Lastensuo bog [45] to examine the uptake of Ni^{2+} and Ag^{+} by viable bacterial cells of these strains in different nutrient and temperature conditions. Incubation times up to 14 days were used to allow active bioaccumulation processes to occur, in addition to faster biosorption processes, in these relatively slowly growing boreal strains. For example, Ni is known to be taken up, in addition to biosorption onto cell walls, into prokaryotic cells by two types of high-affinity transport; through ABC-type transporters and by mechanism that makes use of permeases best described in *Escherichia coli, Ralstonia eutropha, Helicobacter pylori* and *Rhodococcus rhodochrous* J1 [43]. We also determined the uptake of Ni^{2+} and Ag^{+} by the peat profile, from which the bacteria were isolated, and used this information together with the data obtained from the bacterial uptake experiments to estimate the impact of isolated bacterial strains for both Ni^{2+} and Ag^{+} uptake in acidic bog environment in *in situ* conditions.

2. Materials and Method

2.1. Description of the site

Our sampling site, Lastensuo bog, is located on the western coast of Finland. This raised, ombrotrophic bog is surrounded by hummocky till soils and has a surface area of 440 ha [46,47]. The maximum thickness of the peat layer in the middle part of the bog is approximately six meters and below the peat layers a clay layer, derived from former seabed, is found. Mainly in the middle parts

of the bog, gyttja is found on top of this clay layer. Our sampling site is situated on the middle part of the bog, on the area on which mainly four mire types are found: treeless or near-treeless *Sphagnum fuscum* bog, *Sphagnum fuscum* pine bog, ridge hollow pine bog and hollow bog [46]. The main peat types on this area include *Sphagnum* peat, sedge-moss peat, sedge peat and few-flowered sedge [46]. Based on radiocarbon dating peat accumulation on this area started 5300 years ago and the general average peat accumulation has been 1.08 mm/a [46].

2.2. Sampling

A core sample was collected from the middle part of the Lastensuo bog (61 °17′ 31″, 21° 50′ 22″, WGS84 coordinate system) and samples from seven different bog layers: 0.5–1.0 m, 1.5–2.0 m, 2.5–3.0 m, 3.5–4.0 m, 4.5–5.0 m, 5.5–6.0 m and 6.5–7.0 m were obtained. Peat samples were collected using a Russian peat corer with a nest length of 50 cm and diameter of 15 cm. Surface moss (mainly *Sphagnum* spp.) was also collected. The layers from 0.5 to 5.0 m consisted of peat with variable degree of decomposition. The layer from 5.5–6.0 m was gyttja and the lowest layer from 6.5–7.0 m was light grey clay. The samples were taken aseptically in 50 ml sterile centrifugal tubes, Parafilm was attached around the caps and the tubes were sealed in plastic, brought to the laboratory in cooling bags and stored frozen at −18 ℃. The samples were thawed immediately before use and used as such.

The temperature of each bog layer was recorded immediately after the core sample was taken to the surface in early summer, 2nd of June 2015. In the surface layer a temperature of 10.3 ℃ was measured. In lower layers, the temperature was relatively constant, with average of 6.6 ℃. In May 2015 the average temperature at the west-coastal region of Finland (Seinäjoki Pelmaa region) was approximately 9 ℃ and in June 12 ℃ [48]. Typical average temperature in this region in July is around 16–20 ℃ [48].

2.3. Bacterial isolates

The isolation and identification of the bacterial isolates used in this study has been described in detail in Lusa et al. [45]. Shortly, the bacterial strains were isolated from the peat of Lastensuo Bog in June 2013 [45]. The strains were identified by 16S rRNA gene sequencing to belong to the genera *Pseudomonas* (isolates PS-O-L and T5-6-I), *Rhodococcus* (isolate B6-7-CB), *Paenibacillus* (isolates B6-7-W and V0-1-LW) and *Burkholderia* (isolate K5-6-SY) as described in [45] and the sequences were deposited in Genebank under accession numbers KP100420–KP100425. Three of these isolates (*Pseudomonas* T5-6-I, *Pseudomonas* PS-0-L and *Burkholderia* K5-6-SY) were stained Gram negative and the other three (*Paenibacillus* B6-7-W, *Paenibacillus* V0-1-LW and *Rhodococcus* B6-7-CB) Gram positive [45].

2.4. Bacterial culture conditions and preparation of biosorption experiments

Isolated bacterial strains were cultured aerobically on sterile PCA growth plates (PCA, Merckoplate®) at 20 ℃ in the dark and the colonies were moved onto new plates weekly. A batch method using radioactive tracers was used to determine the uptake of Ni^{2+} and Ag^{+} by the bacteria. The uptake tests for triplicate reactions were done using ^{63}Ni (carrier 2 pg Ni(II)/Bq) and ^{110m}Ag

(Ag(I)CN form, no carrier) tracers in two different liquid media (A and B). Medium A comprised 1% Tryptone in which 0.5% NaCl was added and medium B 1% Yeast extract in which 0.5% NaCl was added. Bacterial colonies from the PCA plates were moved into sterile water using a sterile loop and added until the turbidity corresponded to a McFarland standard nro 6, which corresponds to an approximate cell density of 18×10^8 CFU / mL. The suspensions were weighted and 2 mL of this suspension was added to 5 mL of medium A or B, after which 200 Bq of ^{63}Ni or ^{110m}Ag per suspension was added. For experiments with ^{63}Ni the suspensions were incubated for 1, 3, 7 or 14 days at +4 ℃ or +20 ℃ in the dark. For ^{110m}Ag an incubation period of seven days was used and the samples were incubated in the dark. After incubation the suspensions were filtered through a 0.2 µm sterile membrane filter and the activity of the resulting solutions was measured using a NaI(Tl) -gammaspectrometer (Wizard® automatic gammacounter, PerkinElmer). In addition, suspensions without added bacteria were prepared accordingly and measured to assure that no sorption of Ni^{2+} or Ag^+ occurred on laboratory equipment, filters or nutrient broth solutions. The uptake of Ni^{2+} and Ag^+ by bacterial cells was calculated from the difference between initial and final (after filtration) Ni^{2+} and Ag^+ concentration in the solution and expressed as per cent (%) removed.

2.5. Estimation of the proportion of biouptake of the total uptake of Ni^{2+} and Ag^+ by the bacterial isolates in the different layers of Lastensuo bog

The proportion of biouptake of the total uptake by bacteria examined in this study in the different layers of Lastensuo bog was estimated based on the uptake of Ni^{2+} and Ag^+ by fresh moss, peat, gyttja and clay samples (from now on called overburden samples) and the size of the bacterial communities of the Lastensuo bog [49]. For these calculation the uptake of Ni^{2+} and Ag^+ by fresh overburden samples was determined using a batch method (see [50]) as follows: 0.5 g of each sample were weighted into a sterile 50 ml centrifuge tube and 25 ml of simulated bog water (Table 1) containing 200 Bq/sample of ^{63}Ni or ^{110m}Ag tracer was added to the tubes. The samples were incubated for 7 days in the dark, under constant stirring in an over-head shaker (10 rpm), which after the samples were filtered through a 0.2 µm syringe filter (Supor membrane filter, Pall Corp., Port Washington, NY, USA) and the solution was used for gamma spectrometric determination of ^{63}Ni or ^{110m}Ag activity.

Table 1. Simulated bog water used in the uptake experiments with fresh overburden samples as well as in recolonization experiments with Ni. For more information of the simulated bog water see [50].

	Simulated bog water	
	mg/L	meq/L*
Na	3.91	0.17
Mg	0.47	0.04
K	2.03	0.05
Ca	1.98	0.10
Cl	5.34	0.15
NO_3	2.40	0.04
SO_4	8.17	0.17

*meq = milliequivalents

The uptake of Ni^{2+} or Ag^{+} was calculated as the difference between initial and final activity in the solution and expressed as distribution coefficients, K_d (L/kg DW) (see [50]), which were calculated using equation

$$K_d = [(A_i - A_f) / A_f] \times [V(L) / m(kg)] \quad (1)$$

where A_i is the initial ^{63}Ni or ^{110m}Ag activity concentration (Bq/L), A_f is the final activity concentration of the solution (Bq/L), V is the solution volume (L), and m is sample mass (kg DW). All calculations were performed using dry mass determined at 105 ℃. In addition, the average biouptake of the bacterial strains was calculated in the same manner as for the uptake for overburden samples and expressed as K_d. In these calculations m is the bacterial dry mass at t = 0 (kg). Initial mass added was used in the K_d calculations, as the difference in the uptake between different bacteria over the same time period was to be studied. The proportion (P(%)) of Ni^{2+} and Ag^{+} uptake by bacteria was thereafter calculated using equation.

$$P(\%) = (N_L \times m_B \times K_{db}) / K_{dL} \times 100\% \quad (2)$$

where N_L is the bacterial number of the layer ($1/kg^{-1}$), m_B is the mass of bacteria (kg DW), K_{db} is the average uptake of bacteria at +20 ℃ (L/kg DW) and K_{dL} is the average uptake of overburden samples (L/kg DW). Uptake of Ni^{2+} and Ag^{+} by overburden samples was deduced from the experiments done using unsterilized moss, peat, gyttja and clay samples. For the calculations bacterial mass of 0.28 pg DW [51] was used. The bacterial numbers were previously determined from the moss, peat, gyttja and clay samples collected in 2013 [49], that were used in the present study and bacterial numbers of 2.5×10^{10} g^{-1} DW in the moss and gyttja layers, 5×10^{9} g^{-1} DW in the peat layer and 2×10^{9} g^{-1} DW in the clay layer [49] were used in the calculations.

2.6. Uptake of Ni^{2+} in sterilized samples after recolonization with bacteria

The effect of microbes on the uptake of Ni^{2+} in the environment was examined using bacterial and microbial "inoculated" moss, peat, gyttja and clay (overburden) samples. These inoculated samples were prepared by adding isolated bacterial strains or microbial extract from peat or clay to the overburden samples sterilized by gamma irradiation. Change in the removal of Ni^{2+} from the bog water solution was compared with the sterilized overburden samples. This was done to examine whether Ni^{2+} uptake could be reestablished by restoring microbial activity. For these experiments 0.25 g of each of the fresh overburden samples was weighed into a 50 ml sterile centrifuge tube and sent to Scandinavian Clinics Estonia OÜ for gamma irradiation. The samples were irradiated two times; the second irradiation was done 7 days after the initial irradiation. The total dose was 96.0 kGy ± 5%. The microbial extract was prepared from fresh peat (0.5–1.0 m) and clay (6.5–7.0 m) samples by adding fresh sample to sterile MilliQ water in a mass-to-volume proportion of 1:1. The sample was incubated at +20 ℃ for five days in the dark. After incubation peat/clay was allowed to settle and the supernatant containing the microbes was removed by pipetting. Thereafter, 2 mL of the supernatant i.e. the obtained microbial extract or 2 mL of similar bacterial solution as for the experiments with broths A or B and 12.5 mL of sterile bog water solution (Table 1) were used for the recolonization experiments of sterilized peat and clay samples. 200 Bq of ^{63}Ni tracer was added and the samples were further incubated for 7 days under constant stirring, which after the samples were filtered through a 0.2 μm syringe filter (Supor membrane filter, Pall Corp., Port Washington, NY,

USA) and the solution was used for the gamma spectrometric determination of ^{63}Ni activity. The uptake was expressed as the uptake percentage (%) calculated from the difference between initial and final activity in the solution. The uptake of Ni^{2+} from the solution in the recolonized samples was compared to merely sterilized samples with only sterile bog water as well as to un-irradiated fresh samples.

2.7. Statistical analyses

To study the statistical difference between the different growth conditions i.e. the difference between nutrient broths A and B and the temperatures +4 ℃ and +20 ℃ the analysis of variance was performed using OriginPro 8.6 (OriginLab®) and one-way ANOVA at the $p < 0.05$ level. Analysis of variance was done for all studied bacteria separately for temperature and nutrient broth. In addition, the ANOVA analysis for the statistical differences in the uptake between different bacteria and bacterial groups (*Pseudomonas*, *Paenibacillus* and *Burkholderia/Rhodococcus* and $Gram^+$ versus Gram− bacteria) was done.

3. Results

3.1. Uptake of Ni^{2+} by the bacterial isolates in nutrient broth

The uptake of Ni^{2+} by the bacterial isolates of *Pseudomonas* (PS-0-L and T5-6-I), *Rhodococcus* (B6-7-CB), *Paenibacillus* (V0-1-LW and B6-7-W) and *Burkholderia* (K5-6-SY) varied depending on the incubation temperature and nutrient source (broths A and B or sterilized peat) (Figure 1. and 2.). For all studied bacteria Ni^{2+} uptake was found significantly higher at +20 ℃, compared to the uptake measured at +4 ℃. At the latter temperature the average Ni^{2+} uptake of all bacteria in both nutrient broths (A and B) after 7 days of incubation was only 2.3 ± 2.3% while at +20 ℃ the corresponding value was 16.1 ± 8.2%.

Highest uptake for Ni^{2+} was recorded for *Pseudomonas* PS-0-L, for which the maximum uptake of 48% was seen in medium A (1% Tryptone) after 14 days incubation. Moderately high uptake for *Pseudomonas* PS-0-L was also observed in medium B (1% Yeast extract), where the uptake was 35% and 34% after 7 and 14 days incubation, respectively. In addition, for *Paenibacillus* B6-7-W and *Rhodococcus* B6-7-CB moderately high uptake of 26% and 33%, respectively, was observed in medium B. For *Paenibacillus* B6-7-W highest uptake was observed in medium B and for *Rhodococcus* B6-7-CB in medium A after 7 and 14 days of incubation, respectively.

For *Paenibacillus* V0-1-LW, *Pseudomonas* T5-6-I and *Burkholderia* K5-6-SY somewhat weaker uptake for Ni^{2+} was observed, with uptake between 11–16%.

Based on the analysis of variance (ANOVA), the difference in the Ni^{2+} uptake at the two different incubation temperatures (4 ℃ and 20 ℃) was statistically significant ($F_{crit} = 4.04$, $F = 25.5$, $p < 0.01$). For Ni^{2+} uptake no statistically significant difference between the two nutrient broths, 1% Tryptone and 1% Yeast extract ($F_{crit} = 3.99$, $F = 0.10$, $p = 0.75$), nor between different bacterial groups ($F_{crit} = 3.20$, $F = 0.13$, $p = 0.87$) or Gram+/Gram− bacteria ($F_{crit} = 4.05$, $F = 1.39$, $p = 0.25$) were found.

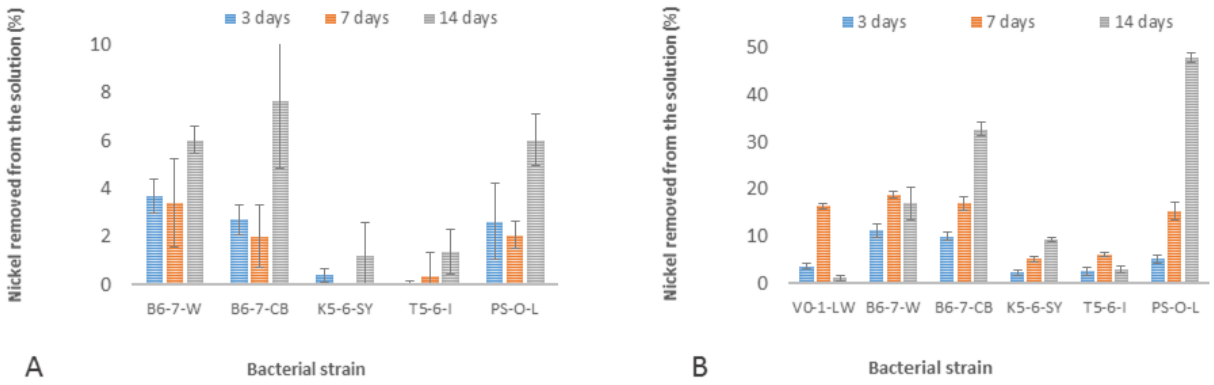

Figure 1. Uptake (%) of Ni^{2+} from 1% Tryptone solution by isolates of *Pseudomonas* (PS-0-L, T5-6-I), *Rhodococcus* (B6-7-CB), *Paenibacillus* (V0-1-LW, B6-7-W) and *Burkholderia* (K5-6-SY). A) 4 ℃, B) 20 ℃. In A) the uptake of $Ni^{2+}$by V0-1-LW was not determined. The error indicated is the standard deviation of three parallel determinations.

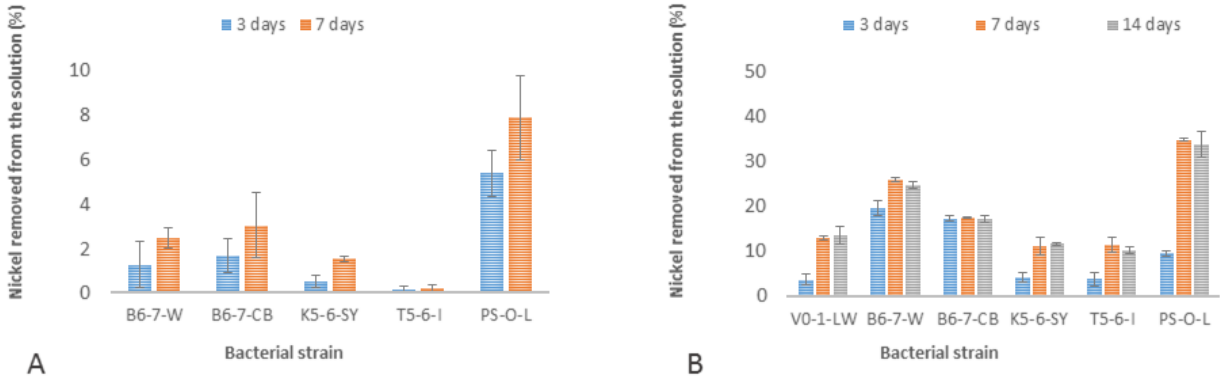

Figure 2. Uptake (%) of Ni^{2+} from 1% Yeast solution by isolates of *Pseudomonas* (PS-0-L, T5-6-I), *Rhodococcus* (B6-7-CB), *Paenibacillus* (V0-1-LW, B6-7-W) and *Burkholderia* (K5-6-SY). A) 4 ℃, B) 20 ℃. In A) the uptake of Ni^{2+} after 14 days incubation or by V0-1-LW was not determined. The error indicated is the standard deviation of three parallel determinations.

3.2. Uptake of Ni^{2+} in bacterial and microbial sterilized and inoculated bog samples

The sterilization of overburden samples by gamma irradiation decreased total Ni^{2+} uptake in all studied overburden layers (moss, peat, gyttja and clay) (Figure 3) and the difference between unsterilized and sterilized samples was found statistically significant using ANOVA (F_{crit} = 4.2, F = 10.8, $p < 0.01$). It was assumed, that the difference between uptake in sterilized samples and unsterilized overburden samples was mainly due to the microbial interactions. In fresh, unsterilized overburden sample there are multiple interactions, abiotic and biotic, which can affect the total Ni^{2+} uptake. These include biouptake as well as various oxidation/reduction type reactions, which can be induced by microbes. The abiotic processes include direct ion exchange and sorption on the organic

(e.g. carboxylic groups found in soil organic matter) and inorganic materials (mineral fraction) found in the sample. In sterilized sample, the microbial processes are eliminated, but abiotic processes remain including dead biomass. Therefore, it can be assumed that this difference between sterilized and unsterilized samples results from the active/living microbiota.

Adding the isolated bacterial strains or microbial extract to sterilized overburden samples increased the uptake of Ni^{2+} in all examined samples, although the efficiency depended on the added bacterial strains or microbial extracts (Figure 3). The greatest removal of Ni^{2+}, compared to the sterilized sample, was observed after addition of microbial extract from peat to the sterilized surface moss sample in which case the removal increased from 56% to 88%. Similarly, as microbial extract from peat was added to the sterilized peat sample, the removal of Ni^{2+} increased from 66% to 88%. In the gyttja sample the increase in Ni^{2+} uptake induced by the microbial extract was low, from 65% to 74%. The microbial extract from clay had no significant effect on the Ni^{2+} removal.

Burkholderia K5-6-SY had the most prominent effect of the bacterial strains on the Ni^{2+} removal from simulated bog water. In all studied overburden samples this bacterium increased the removal of Ni^{2+} most compared to the other bacterial strains. The most significant change in the Ni^{2+} removal was observed in the peat sample, in which Ni^{2+} removal increased (similarly to the peat microbial extract) from 66% to 88%. In the clay sample, a significant increase from 12% to 20% was also observed after addition of *Burkholderia* K5-6-SY.

When Ni^{2+} uptake in sterilized overburden samples with added bacteria or microbial extract were compared with unsterilized, pristine overburden samples, it was found that addition of microbial extract from peat to the sterilized surface sample restored Ni^{2+} uptake to the level found in the pristine sample. As microbial extract from peat or *Burkholderia* K5-6-SY was added to sterilized peat, Ni^{2+} uptake was somewhat higher (88%) than in pristine samples (81%).

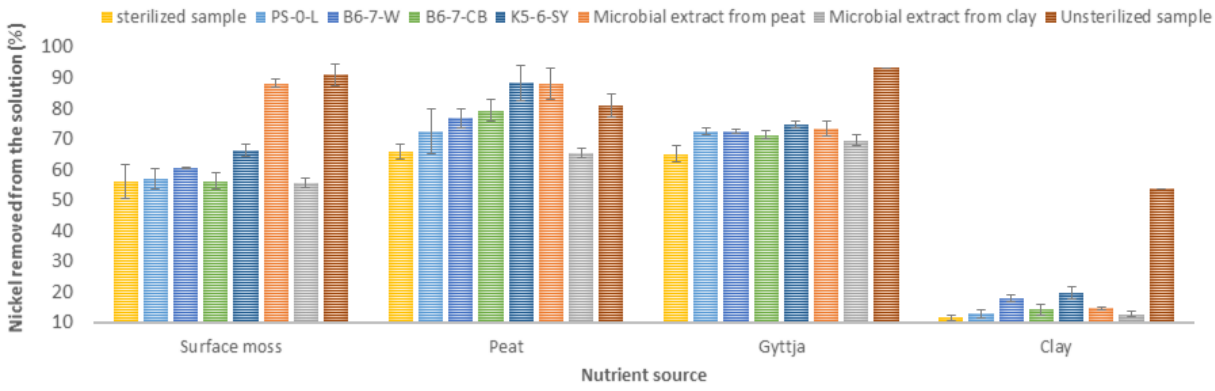

Figure 3. Uptake (%) of Ni^{2+} from sterilized overburden solution after addition of *Pseudomonas* (PS-0-L, T5-6-I), *Rhodococcus* (B6-7-CB), *Paenibacillus* (V0-1-LW, B6-7-W) and *Burkholderia* (K5-6-SY) strains as well as microbial extracts from peat and clay layers of Lastensuo bog.

3.3. Uptake of Ag^+ by the bacterial strains in different nutrient broth

The uptake of Ag^+ by the bacterial isolates depended on the incubation temperature and nutrient source (broths A and B) (Figure 4). As for the Ni^{2+} uptake, the Ag^+ uptake was lower at lower

incubation temperature although the effect was less prominent; the average uptake of Ag^+ at +4 ℃ was 18% and at +20 ℃ 30% after seven days incubation. Furthermore, for all studied bacteria Ag^+ uptake was significantly higher than the Ni^{2+} uptake. At +4 ℃ the Ag^+ uptake was on average eight-fold that observed for Ni^{2+} uptake and at +20 ℃ the Ag^+ uptake was two times higher than the Ni^{2+} uptake.

Highest Ag^+ uptake was observed for *Paenibacillus* V0-1-LW and *Pseudomonas* PS-0-L, where the maximum uptake was 73% and 49%, respectively in 1% Yeast extract at +20 ℃.

Paenibacillus B6-7-W, *Pseudomonas* T5-6-I, *Rhodococcus* B6-7-CB and *Burkholderia* K5-6-SY showed a slightly weaker uptake of Ag^+, with a maximum uptake between 15–31% in 1% Tryptone at +20 ℃. For *Pseudomonas* T5-6-I there was no difference in Ag^+ uptake between the two incubation temperatures in 1% Tryptone. For all other studied bacteria Ag^+ uptake was on average two times higher at +20 ℃, compared to the uptake at +4 ℃ in both nutrient broths.

The most prominent difference in the maximum Ag^+ uptake, was observed between the two *Paenibacillus* sp. strains V0-1-LW and B6-7-W. The maximum uptake in the former (73%) was four times higher than the maximum uptake observed in the latter (17%). A particularly clear difference between these two strains of *Paenibacillus* was observed at +4 ℃ in 1% Yeast extract, where the Ag^+ uptake for *Paenibacillus* V0-1-LW was 54%, but for B6-7-W only 3.5%.

For Ag^+ uptake, no statistically significant differences were found between different incubation temperatures (F_{crit} = 4.30, F = 3.06, p = 0.09), nutrient broths (F_{crit} = 4.30, F = 0.16, p = 0.69), bacterial groups (F_{crit} = 3.47, F = 2.00, p = 0.16) or Gram+/Gram− bacteria (F_{crit} = 4.30, F = 0.12, p = 0.73).

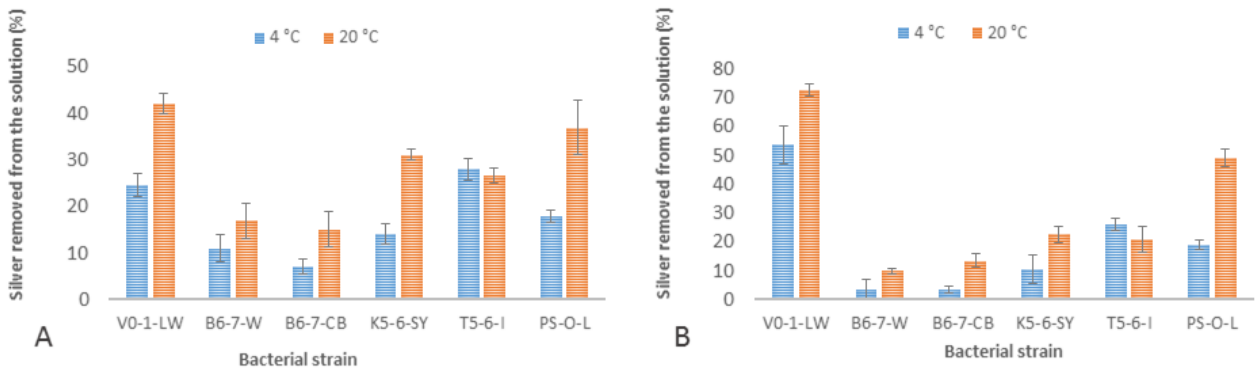

Figure 4. Uptake (%) of Ag^+ from A) 1% Tryptone and B) 1% Yeast extract solutions by isolates of *Pseudomonas* (PS-0-L, T5-6-I), *Rhodococcus* (B6-7-CB), *Paenibacillus* (V0-1-LW, B6-7-W) and *Burkholderia* (K5-6-SY) at +4 ℃ and +20 ℃. The error indicated is a standard deviation of three parallel determinations.

3.4. Estimation of the total biouptake proportion of Ni^{2+} and Ag^+ by Pseudomonas, Rhodococcus, Paenibacillus and Burkholderia in the different layers of Lastensuo bog

Ni^{2+} uptake by the fresh bog samples was highest in the gyttja sample. In this layer the distribution coefficient (K_d value) for Ni^{2+} uptake was 19 900 L/kg DW (Table 2). In the moss and peat layers somewhat lower K_d values of 13 060 L/kg DW and 7800 L/kg DW, respectively, were

measured. The clay sample showed the lowest uptake, with K_d value of 190 L/kg DW. For Ag^+ the K_d values in moss, peat, gyttja and clay layers were 1700 L/kg DW, 21 800 L/kg DW, 17 800 L/kg DW and 10 700 L/kg DW, respectively. For the bacteria the average uptake at +20 ℃, expressed as K_{db} were 440 L/kg DW for Ni^{2+} and 5620 L/kg DW for Ag^+.

Using equation 2, we estimated that the proportion of Ni^{2+} uptake by the added *Pseudomonas* (PS-0-L, T5-6-I), *Rhodococcus* (B6-7-CB), *Paenibacillus* (V0-1-LW, B6-7-W) and *Burkholderia* (K5-6-SY) accounted for approximately 0.02% of the total sorption in the moss layer, 0.01% in the peat layer, 0.02% in the gyttja layer and 0.1% in the clay layer of the bog. For Ag^+ the corresponding values were 2.3% in the moss layer, 0.04% in the peat layer, 0.2% in the gyttja and 0.03% in the clay layer.

Table 2. The distribution coefficients (K_d values) in fresh surface moss, peat, gyttja and clay samples of Lastensuo bog.

	Ni uptake L/kg DW	Ag uptake L/kg DW
Surface	13 100	1 700
Peat	7 800	21 800
Gyttja	19 900	17 800
Clay	190	10 700

4. Discussion

^{59}Ni and ^{108m}Ag are among the most important radionuclides in the long-term biosphere safety assessments of spent nuclear fuel disposal due to their long half-life, rapid release from the fuel after contact with groundwater and rather easy migration through the bedrock [13,15,26]. In addition, the stable isotopes of both of these metals are highly toxic in the environment and potentially accumulate in the food chains [9,23].

In recent years, research has focused on the use of microorganisms in the removal and potential salvage of metals and radionuclides from industrial waste waters [27]. However, these studies have focused on the use of algae and fungi, like *Oedogonium hatei, Sphaeroplea Algae, Aspergillus niger* and *Trichoderma viride* [1,52–54] in Ni bioremoval. Fewer studies of Ni^{2+} biosorption on bacteria, such as *Bacillus* sp. and *Pseudomonas fluorescens* are available [27,35]. In the case of Ag^+, biosorption studies have been focused on the use of e.g. cyanobacteria (*Spirulina platensis* [55,56]) and baker's yeast (*Saccharomyces cerecisiae* [57,58]). Of bacteria *Magnetospirillum gryphiswaldense, Bacillus licheniformis, Streptomyces* spp., *Arthrobacter oxidas* and *A. globiformis* have been used [56,59]. The bacteria used in the Ni^{2+} and Ag^+ biosorption studies have been isolated from e.g. electroplating sludges and effluents (e.g. *P. fluorescens*, *Bacillus* sp.), gold mine (*B. licheniformis*), basalts rocks (*A. oxidas* and *A. globiformis*) and the rhizosphere of soybeans (*Streptomyces* spp.) [27,35,56,59]. Bioremoval of heavy metals by microorganisms has however been reported to be dependent, in addition to the chemical nature of heavy metal, on the species of microorganism as well as environmental conditions [60].

Acidic, ombrotrophic bogs are unique habitats that are widely distributed in cold, temperate regions found in boreal ecosystems of the Northern hemisphere. These areas, with high importance for biodiversity, have distinctive microbial populations [49,61–64], affected by low nutrient levels,

acidic water and changing seasons from cold, snow-covered winters to temperate summers. However, to our knowledge, studies on the biosorption of heavy metals on bacteria, isolated from boreal environment are scarce.

In this study all tested bacterial strains removed both Ni and Ag from the solution. The uptake efficiency was however affected by incubation temperature and nutrient source. A higher uptake of Ni and Ag was found at +20 ℃ than at +4 ℃. These two temperatures were chosen as they can be expected to be found in a bog in the temperate climate prevailing in Finland, where the temperature of deeper bog layers is typically around +4 ℃ and the upper layers may experience +20 ℃ during the summer months (July and August). The effect of the nutrient source clearly affected the uptake efficiency of the different metal ions and the highest uptake of Ni was detected in 1% Tryptone broth whereas for Ag uptake the 1% Yeast extract broth was most efficient. This may be due to the different nutrient requirements by the bacterial isolates, since *Pseudomonas* sp. PS-0-L removed 48% Ni from the solution when 1% Tryptone was used and only 35% when 1% Yeast extract was used. In contrast, the most efficient Ag remover, *Paenibacillus* sp. V0-1-LW removed 73% Ag from the solution when 1% Yeast extract was used, but only 42% when 1% Tryptone was used.

Each bacterial strain had unique uptake properties. The most significant difference in the maximum uptake of Ni, was observed between the two *Pseudomonas* sp. strains, as the maximum uptake in PS-0-L was 76% higher than that observed in T5-6-I. This is not surprising as the two isolates also differed in the utilization patterns of different substrates tested earlier by the RapID system [45]. For the two *Paenibacillus* strains V0-1-LW and B6-7-W the difference in Ni^{2+} uptake was considerably lower; the maximum uptake in V0-1-LW was 63% of the maximum uptake observed in B6-7-W. For Ag^{+}, the most prominent difference in the maximum uptake was observed between two strains of *Paenibacillus* sp., 73% and 17% for V0-1-LW and B6-7-W, respectively, especially at +4 ℃ in 1% Yeast extract broth. Thus, our results indicate that the uptake of Ni and Ag is dependent on the strain of bacteria, as well as on the species. The difference in the Ni and Ag uptake between the different bacterial species may be related to the chemical properties of metal sorbates and the properties of functional groups and cell wall structures of each bacterium. For both Gram negative and Gram positive bacteria the most important binding sites found in the cell wall are carboxyl (-COOH), phosphoryl ($-PO_3^{2-}$) and sulfhydryl (-SH) sites, and these groups differ in their affinity and specifity for metal binding [65,66]. Uptake on these sites is accompanied by displacement of protons and therefore depends on the degree of protonation, which in turn is affected by the pH value. Typically, bacterial cells exhibit buffering capacity, which for example in *B. subtilis* and *S. oneidensis* ranges from approximately pH 3 to pH 9 [66]. This kind of buffering capacity is a result of distinct acidic sites located on the cell walls [66] and is of importance in changing environmental conditions. In addition to the biosorption on the functional groups on cell walls, accumulation of intra- or extracellular precipitates is also possible [27] For example, for *P. fluorescens* 4F39 accumulation of dense Ni structures on the cell wall, corresponding to two orientations of $Ni(OH)_2$ crystals, has been demonstrated [27].

Biosorption is possible both on living and dead cells, but mechanisms in which heavy metals and radionuclides are taken up using active bioaccumulation processes are present only in living cells [43,67,68]. For example, Ni^{2+} is known to be taken up into prokaryotic cells via ATP-binding cassette transporters (ABC-type transporters) and Ni-specific permeases [43]. In these mechanisms, the Ni^{2+} ion is specifically incorporated into Ni-dependent enzymes like urease, NiFe-hydrogenase, carbon monoxide dehydrogenase, methyl coenzyme M reductase, certain superoxide dismutases,

some glyoxylases and methylenediurease [43]. Incorporation usually occurs via complex assembly processes and requires accessory proteins and additional non-protein components [43]. These mechanisms are typically significantly slower than direct biosorption onto cell walls. In our samples, it was seen that uptake of Ni^{2+} increased with longer incubation times (7 and 14 days), compared to shorter incubation times (1 and 3 days). One explanation for this increase, in addition to slower bioaccumulation processes, is a possible change in the cell numbers and activities of the bacterial population in the sample over time. Over longer period of time the population of the bacteria initially added to the sample increases up to a certain point, which after the population starts to decrease, as can be seen as a decrease in the Ni^{2+} uptake with longer incubations. It was observed that after one day of incubation at +20 ℃, Ni^{2+} uptake was on average only 3.3% (range 0.9–7.7%). This uptake can be assumed to be mostly a result from direct biosorption on cell walls. After 3 days incubation the average uptake increased to 7.8% (range 2.3–20%). After longer incubation periods the average uptake was significantly higher, 16% (range 5.3–35%) and 19% (range 1.2–48%), after 7 and 14 days, respectively. The major part of this slow accumulation is expected to result from slow bioaccumulation processes involving active transport by living cells and also changes in extracellular matrix can affect the uptake. We assume that under *in situ* conditions of the bog, these slow processes are an important part of Ni^{2+} retention. It was estimated, based on the sorption experiments done with fresh overburden samples, that in *in situ* conditions of the bog the bacterial uptake of Ni^{2+} accounts for approximately 0.02% of the total sorption in the moss layer, 0.01% in the peat layer, 0.02% in the gyttja layer and 0.1% in the clay layer of the bog. This estimation was however done using average uptake of Ni^{2+} of only six isolated bacterial strains, which represented a minority of the total bacterial population [49], not to mention the whole microbial population of the bog. It should also be noted that these calculations are rough estimates of the total biouptake proportion, as the total metal sorption and bioaccumulation may depend on the physiological status of the bacterial populations and therefore the number of bacteria might not correlate directly with metal sorption. Therefore, also another approach, namely the samples re-inoculated with bacterial and microbial extracts, was used in this study. It was found that sterilization of the overburden samples in the surface, peat and gyttja layers of the bog decreased Ni^{2+} retention from an average of 80% to an average of 50%, with most prominent decrease from 91% to 56%, observed in the surface moss layer. Furthermore, it was observed that recolonization of the sterilized overburden samples with isolated bacteria and microbes extracted from fresh overburden samples, restored the retention of Ni^{2+} in the sterilized overburden samples to the same level as was observed in the unsterilized ones.

Previously Ni uptake has been studied using *Pseudomonas fluorescens* [27]. As we compared the Ni uptake of *Pseudomonas* PS-0-L of our study to the Ni uptake of *Pseudomonas fluorescens*, it was found that the uptake by *Pseudomonas* PS-0-L is approximately only 1% of the uptake observed for *Pseudomonas fluorescens* [27]. However, previously we used the same bacterial isolates that were used in this study to examine their uptake capacity for iodide (I^-) [45], selenite (SeO_3^{2-}) [69] and cesium (Cs^+) [70] and it was observed that all these bacteria were able to remove also I^-, SeO_3^{2-} and Cs^+ from the solution. The average uptake capacity of all five studied ions followed the sequence $Ag^+ > SeO_3^{2-} > Ni^{2+} > I^- > Cs^+$. The uptake was however variable and depended on the incubation conditions and nutrient broths used. For example, in addition to the broths used in Ni^{2+} and Ag^+ experiments, we also used 0.5% Peptone +0.25% Yeast extract in the I^- uptake experiments [45]. It was found that in this broth *Paenibacillus* V0-1-LW removed I^- from the solution with very high efficiency and in practice all I^- was removed from the solution. In the other used broths, I^- removal

was however significantly lower (<10%). When the uptake of I^-, SeO_3^{2-} and Cs^+ was compared to the uptake of Ni^{2+} and Ag^+ it was found that the same bacterial isolates that had high uptake capacity for Ni^{2+} (*Pseudomonas* PS-0-L, max 48%) and Ag^+ (*Paenibacillus* V0-1-LW, max 73%), also had the highest capacities for I^- (*Paenibacillus* V0-1-LW, max 100%), SeO_3^{2-} (*Pseudomonas* PS-0-L, max 65%) and Cs^+ (*Paenibacillus* V0-1-LW, max 12%) [45,69,70]. Also, when the experiments using sterilized overburden samples and added bacteria were compared, it was found that *Burkholderia* K5-6-SY had the most prominent effect on both the removal of Ni^{2+} and SeO_3^{2-}.

It appears that *Paenibacillus* V0-1-LW, *Pseudomonas* PS-0-L and *Burkholderia* K5-6-SY have vast ability to utilize a large number of elements in their metabolism, or on the other hand they may be characterized by diverse cell wall structures, which can serve as biosorption sites for many different materials. Presumably, e.g. Ni^{2+} and SeO_3^{2-} uptake mechanisms differ from each other quite clearly, taking into account the significant differences in the chemical properties of these substances.

5. Conclusion

Bacteria isolated from an acidic, nutrient-poor bog were able to remove both Ni^{2+} and Ag^+ from the solution and the uptake efficiency depended on temperature, nutrient source and bacterial strain. Even though the isolated bacteria belonged to the minority of the whole bacterial population of the bog, the fact that they all were able to remove both Ni^{2+} and Ag^+ from solution indicates that Ni^{2+} and Ag^+ uptake is a common feature for bacteria found in this environment. Based on the biouptake experiments and experiments conducted with sterilized and bacterial/microbial re-inoculated overburden samples, it is most likely that bacteria are capable to influence the geochemical behaviour of Ni^{2+} and Ag^+ in the northern, boreal environment also under *in situ* conditions. As our data indicates that uptake continues over longer periods of time (incubation times from 7 to 14 days), it is assumed that in addition to direct biosorption processes, also slower, active bioaccumulation processes may be possible. In the future it would be important to identify the active components of the bacterial cell wall that are involved in the bioaccumulation of radionuclides to better understand their retention processes in natural ecosystems.

Conflict of Interest

All authors declare no conflict of interest in this paper.

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