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Sorption behaviour of I^-, SeO_3^{2-} and Cs^+ in an ombrotrophic boreal bog

A study on microbial effects

Merja Johanna Lusa

ACADEMIC DISSERTATION

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Abstract

^{129}I, ^{79}Se and ^{135}Cs are among the most important radionuclides in the biosphere safety assessments of the disposal of spent nuclear fuel. The sorption, retention and migration of these nuclides in the surface environment is of importance when the radiation doses for humans and other organisms in the future is considered. In this doctoral thesis the abiotic and biotic factors affecting the retention of iodide (I^-), selenite (SeO_3^{2-}) and cesium (Cs^+) in a nutrient-poor boreal bog environment were investigated. Batch sorption experiments were used both for the bog layer samples from the surface moss, subsurface peat, gyttja and clay layers of the bog and for bacteria isolated from the bog. The bacteria isolates belonged to four different phyla: *Pseudomonas*, *Rhodococcus*, *Burkholderia* and *Paenibacillus* commonly found in the various environments.

I^- and SeO_3^{2-} retention in the surface moss, peat, gyttja and clay was found to be strongly linked to the microbial activity found in this bog. Sterilization of the surface moss, peat, gyttja and clay samples significantly reduced the retention of both I^- and SeO_3^{2-} and anoxic conditions reduced the sorption of I^-. These results supported the hypothesis that viable microbiota (bacteria/fungi) are necessary for the incorporation of I^- into the organic matter and for the retention of SeO_3^{2-} through microbiotically mediated reduction in the acidic bog environment and that I^- is oxidized into I_2 and/or HIO prior to its incorporation into the organic matter. In the case of SeO_3^{2-} the removal from the solution phase presumably takes place via reduction of SeO_3^{2-} into insoluble Se^0 (and possible further reduction to Se^{2-}, which reacts with iron). Some proportion of abiotic reduction of SeO_3^{2-} in association with sulfide oxidation is possible, but the majority of the reduction is assumed to occur microbiotically. This is supported by the observation that SeO_3^{2-} removal from the solution was at the same level both under oxic and anoxic conditions, but was decreased as samples were sterilized and incubated under oxic conditions. In addition the bacteria isolated from the bog were found to remove both I^- and SeO_3^{2-} from the solution, although the removal was considerably higher for SeO_3^{2-}.

The behaviour of Cs^+ was affected by both abiotic and biotic factors (i.e. pH, clay minerals and bacteria) in the acidic nutrient-poor boreal bog investigated in this thesis. Increase in the pH, increased the sorption of Cs^+ in all studied bog layers and highest sorption was observed in the bottom layer of the bog. In this layer, clay minerals, especially illite, were found. Sterilization of the samples decreased the sorption of Cs^+, but the difference between sterilized and unsterilized samples was not statistically significant. However the bacteria isolated from the bog were found to remove Cs^+ from the solution, though the extend of the removal was significantly lower than that observed for SeO_3^{2-}. In addition implications on the importance of plant uptake and rhizoidosphere effects of *Sphagnum* moss on the Cs^+ retention in the surface layer of the bog were observed.

List of Original Publications

The thesis is based on the following original publications, which are referred in the text by their Roman numerals (I – IV).

I. M. Lusa, M. Bomberg, H. Aromaa, J. Knuutinen, J. Lehto: Sorption of radioiodide in an acidic, nutrient-poor boreal bog: Insights into the microbial impact. *Journal of Environmental Radioactivity* **2015**, 143, 110 – 122.

II. M. Lusa, J. Lehto, H. Aromaa, J. Knuutinen, M. Bomberg: The uptake on radioiodide by *Paenibacillus* sp., *Pseudomonas* sp., *Burkholderia* sp. and *Rhodococcus* sp. isolated from a boreal nutrient-poor bog. *Journal of Environmental Sciences* **2015**, in press.

III. M. Lusa, M. Bomberg, H. Aromaa, J. Knuutinen, J. Lehto: The microbial impact on the sorption behaviour of selenite in an acidic, nutrient-poor boreal bog. *Journal of Environmental Radioactivity* **2015**, 147, 85 - 96.

IV. M. Lusa, M. Bomberg, S. Virtanen, J. Lempinen, H. Aromaa, J. Knuutinen, J. Lehto: Factors affecting the sorption of cesium in a nutrient-poor boreal bog. *Journal of Environmental Radioactivity* **2015**, 147, 22 – 32.

Author's contribution to the publications I – IV:

The author has planned all the experimental work for the publications I – IV and executed the experimental work together with part of the co-authors, except of the 16S rRNA sequencing of the bacterial isolates which was performed at VTT by M. Bomberg. The results in manuscripts I – IV were analysed by the author and the manuscripts I – IV have been written by the author. The IC analyses of bog water samples were performed by the University of Helsinki, Department of Geosciences and Geography, the XRD analyses of the gyttja and clay samples were performed by Stenman minerals AB, Helsinki and DOC (dissolved organic carbon) was determined by the Finnish Forest Research Institute (Metla).

Abbreviations

ABC transporter	ATP-binding cassette transporter
ANOVA	analysis of variance
ATP	adenosine triphosphate
BP	before present
DNA	deoxyribonucleic acid
DOC	dissolved organic carbon
DW	dry weight determined at 105 °C
G-C content	guanine-cytosine content on a DNA molecule
GO	glucose oxidase
GS-Se-SG	glutathione selenitrisulfide
FA	fulvic acid
FES	frayed edge sites
HA	humic acid
HIO	hypoiodous acid
HTP	in sequencing, high-throughput sequencing
HPO	haloperoxidase
IC	ion chromatography
ICP-MS	inductively coupled plasma mass spectrometry
IRF	instant release fraction
K_d	sorption distribution coefficient
L-DOPA	L-3,4-dihydroxyphenylalanine
LOI	loss on ignition at 550 °C, a proxy for organic matter content
MQ water	ultra-pure water
NADPH	nicotinamide adenine dinucleotide phosphate
OYE enzyme	Old Yellow Enzyme, NADPH oxidoreductase
PCA	plate count agar
PCR	polymerase chain reaction

PDTC	2,6-pyridinedicarbothioic acid
pH_{pzc}	pH zero point of charge
PSO	pseudo-second-order kinetic model
R	used as symbol for Pearson's correlation coefficient
R-COOH	carboxylic group
$R\text{-}NH_3$	amino group
rRNA	ribosomal ribonucleic acid
SNF	spent nuclear fuel
SOM	soil organic matter
T3	triiodothyronine
T4	tetraiodothyronine
XRD	X-ray diffraction spectroscopy

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This work would not have been possible without support and assistance of numerous people. First of all, I want to thank my supervisor Jukka Lehto for giving me the opportunity to participate into the Biosphere project, in which the topic of my thesis gradually took shape. Jukka has given me a free hand to modify the topic of my thesis into its current form and allowed me to grow into an independent scientist. I am also greatly indebted to my other supervisor, Malin Bomberg from VTT Technical Research Centre of Finland, who joined us quite in the final phase of my thesis. However without Malin's expertise in the environmental microbiology, the topic would have remained incomplete. I am grateful for our cooperation and I hope it will continue also in the future. It has been very nice to work with you. Professor Brit Salbu from the Norwegian University of Life Sciences is gratefully acknowledged for accepting the role of the opponent for the public examination.

The present and past members and co-authors of the Biosphere research group at the Laboratory of Radiochemistry are acknowledged for the ideas and plenty of data during these years which enabled the publication of the papers included in this thesis. Thank you all! I thank all my fellow Ph.D. students for their friendship and for creating such a nice atmosphere to work in. Special thanks goes to Sinikka Virtanen, Janne Lempinen, Hanna Tuovinen and Jenna Knuutinen for your friendship and a number of nice lunch hours and dinner meetings. The rest of the personnel at the laboratory are thanked for their help in various situations and for a superb environment to work in.

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Ojakkala, August 2015 Merja Lusa

Table of Contents

1. Introduction

Nuclear energy production results in spent nuclear fuel (SNF), which in Finland will be disposed of in a 400 m deep bedrock repository in the crystalline bedrock on Olkiluoto Island, located in south western Finland. The Olkiluoto area was covered by the Scandinavian Ice Sheet during the last glaciation episode in the Pleistocene, until the last deglaciation 17 000 - 10 000 years BP (Kallio 2006, Hyttinen 2012, Björck and Möller 1987). The gradual melting of the Scandinavian Ice Sheet and the glacio-isostatic uplift after the ice retreated, caused the Baltic to undergo several different phases from the Yoldia Sea stage about 11 000 years BP, through the Ancylus Lake and Litorina Sea stages to the Baltic Sea (Kallio 2006). The Olkiluoto Island lay below the water surface level of these stages and rose above the sea level about 3000 – 2500 years BP (Mäkiaho 2005). The post-glacial land-uplift continues on the area, being currently about 6 mm/year (Smellie et al. 2014). Resulting from the land-uplift the Olkiluoto Island will develop into an inland site within 6000 years and during the same time period bogs will be formed in the area (Haapanen et al. 2013). According to the biosphere safety assessment calculations, the first possible releases from the deep spent nuclear fuel repository into the surface biosphere, if some of the nuclear canisters would leak, would be possible at the same time period as the narrow strait between the mainland and Olkiluoto disappears and bogs appear on the area (Posiva 2012). Lastensuo bog, examined in the present work, represents the biotope expected to develop into the area, and has therefore been chosen as an analogue biotope in the biosphere safety assessments of the long-lived radionuclides (Haapanen 2011).

The concept of final disposal of SNF is based on several engineered barriers planned to prevent the release and further migration of radionuclides into the surface biosphere (Hjerpe et al. 2010, Helin et al. 2010). However as the constructed barriers may eventually leak, the estimation on the possible radiation doses to humans in the future is essential. ^{79}Se, ^{129}I and ^{135}Cs are among the most important long-lived radionuclides, as the possible radiation doses for humans through drinking water or food-chains in the future are considered in the biosphere safety assessment calculations (Hjerpe et al. 2010). ^{129}I has been classified as one of the top priority radionuclides in these assessments together with ^{36}Cl and ^{14}C. ^{79}Se and ^{135}Cs belong to the high priority class (Hjerpe et al 2010). Top priority class radionuclides or their progeny nuclides are expected to dominate in the dose caused by the radionuclides potentially released from the repository into the surface biosphere (Helin et al. 2010). For high priority radionuclides the effect on the dose is expected to be significant. The surface environment itself has no safety function in the disposal of SNF, but as the potential harmful radiation risks will occur in the biosphere, understanding of the behaviour of the nuclides causing the highest potential dose is important (Posiva 2012B). This includes sorption and accumulation of these nuclides in the geological, hydrological and biological cycles of the surface environment.

2. Final disposal of spent nuclear fuel and the biosphere safety assessment

The deep crystalline bedrock repository for SNF is based on the KBS-3 model adapted from Svensk Kärnbränslehantering AB (SKB) (Hjerpe et al. 2010, Helin et al. 2010) and the long-term safety of this repository model is based on several barriers. These barriers include the fuel itself (UO_2), the copper canister with a cast iron insert, the bentonite clay protecting the canister, the backfill material of the tunnels and the bedrock of the repository (Raiko 2005, SKB 1983A, SKB 1983B). The conditions in the deep bedrock repository are generally expected to be reducing, under which conditions the dissolution of the uranium fuel itself, UO_2, containing most of the radionuclides is very low. In the copper canisters the cast insert provides mechanical strength and radiation shielding and the copper can resist from corrosion (Raiko 2005). The bentonite clay, which acts as buffer material constitutes both mechanical and chemical protection for the fuel canisters (SKB 1983A). Bentonite has a high capacity to absorb water, leading to considerable swelling. Bentonite also has a high capacity to retain certain nuclides through ion exchange (e.g. cesium) (Sabodina et al. 2006). However, if the waste canisters were to lose their integrity, radionuclides could escape from the repository and according to the different release scenarios used in the biosphere safety assessments the fail in the copper canister could lead to the release of radionuclides from the fuel matrix and their eventual migration through the geosphere into the biosphere (Hjerpe et al. 2010, Vieno and Nordman 1999). A time window of 10 000 years is used in the biosphere safety assessment and the general purpose of the assessment is to determine the radiological consequences of the potential future releases of radionuclides from the deep bedrock repository to humans and other organisms (Posiva 2012, Hjerpe et al. 2010). This includes the modelling of the fate and transport of the radionuclides hypothetically released from the repository to the surface environment as well as describing and assessing the prevailing processes in the surface environment (Hjerpe et al. 2010). According to biosphere modelling calculations the emissions of ^{129}I, ^{79}Se and ^{135}Cs to the wetland acrotelm (the oxic layer at approximately 0.2–0.4 m below the land surface) after a hypothetical release from the canisters would begin to increase after approximately 2500 years after the disposal and to cause a major proportion of the probable dose until the end of the time window of the biosphere assessment 10 000 years from now (Hjerpe et al. 2010). According to the guide lines for the biosphere safety assessment the disposal have to be planned in a way that due to the expected evolution of the barrier system the annual effective dose to the most exposed individual will remain below 0.1 mSv and the average individual doses to larger groups of the public remain insignificantly low (Vieno and Nordman 1999). Currently the average annual radiation dose for Finns is approximately 4 mSv/year, caused mostly by indoor radon and X-ray examinations.

For the modelling purposes, data (e.g. distribution coefficient, K_d, values) concerning the top and high priority nuclides in different natural environments expected to be found in the repository area in the future is essential. The results presented in this thesis may be used in the biosphere assessment concerning the interactions of long-lived nuclides of iodine (I), selenium (Se) and cesium (Cs) present in nuclear waste in the nutrient-poor bog environment with low pH and the effect of bacteria and other microbiota on their behaviour. The primary goal of this study has been to obtain knowledge about the sorption and retention of iodide (I^-), selenite (SeO_3^{2-}) and cesium (Cs^+) in the moss, peat, gyttja and clay layers of the bog and the different factors affecting their behaviour, i.e. depth, pH,

time, temperature and microbiota. In addition the biosorption of I^-, SeO_3^{2-} and Cs^+ on *Pseudomonas* sp., *Burkholderia* sp., *Rhodococcus* sp. and *Paenibacillus* sp. isolated from the Lastensuo bog was studied. The microbial effect on the radionuclide retention and migration was one of the main perspectives of this study as although wetland microbiology has been studied for decades the functional roles of many inhabitants in northern bogs remain unknown.

3. The mire environment and the microbiota inhabiting northern mires

Northern mires are moist environments characterized by their unique capacity to accumulate peat which is sustained by humid climate (Heikkilä and Heikkilä 2002, Malmer 2014, Juottonen et al. 2005). Peat is formed by partial decomposition of mosses and other bryophytes, sedges, grasses, or shrubs and is accumulated predominantly in the oxic acrotelm which has a varying water table level and lateral movement of water (Moore 1989, Malmer 2014, Coccozza et al., 2003). The formation of peat depends on the excess of plant productivity over the respiratory processes of microorganisms in the particular ecosystem (Moore 1989). Peat accumulation is frequently more dependent on the reduced microbial activity rather than to high plant productivity, especially in nutrient-poor bog environments. Hence peat accumulates in environments in which physical conditions serve to reduce the rate at which decomposers can consume the available organic resources. Many factors reduce the respiratory activity of aerobic microbes, such as low oxygen concentration, low pH, low temperature, differences in water content, and the flow and quality of water, as well as the amount of nutrients and these factors vary considerably between different peatland types (Moore 1989, Heikkilä and Heikkilä 2002). Probably the most typical cause for peat formation is the depletion of oxygen associated with waterlogged conditions. This is why peat formation is closely linked with hydrologic factors (Moore 1989). Mires are very diverse habitats and often different mire types can be found next to each other, forming mosaics of different ecological regions (Heikkilä and Heikkilä 2002). Mires can be classified into two main types based on the hydrological conditions (Moore 1989); rheotrophic and ombrotrophic mires. Rheotrophic mires get their nutrients both from rain- and ground-water flow while ombrotrophic mires receive water and nutrients only form rainfall (Moore 1989, Heikkilä and Heikkilä 2002). These two types do not differ only in the amount of water they obtain, but also in the quantity of dissolved and suspended inorganic nutrients and minerals. This causes variations in the vegetation, as the demand of inorganic nutrients, mineral proportion of breeding ground as well as that of organic materials vary between plant species.

In addition to the two main mire types, mires have been classified in multiple subclasses including bogs, fens and forested peatlands. Most bogs are more acidic and poorer in nutrients than fens (Malmer 2014). In practice, bogs are distinguished from fens based on fen indicator vegetation (exclusive fen plants) such as *Menyanthes trifoliata* and several species of *Carex* are used (Malmer 2014). The mire examined in present work, Lastensuo, located on the western coast of Finland, represents an ombrotrophic, nutrient-poor boreal bog (Figure 1). Different mire types are found in the 440 ha area comprising the Lastensuo bog, and in the center parts of the bog treeless or near-treeless *Sphagnum fuscum* bog, *S. fuscum* pine bog, ridge hollow pine bog and hollow bog dominate (Mäkilä and Grundström 2008). Towards the margins of the bog, the mire types change through low sedge bog and cotton grass pine bog to tall sedge pine fen and forested peatland. The main peat types found in Lastensuo include *Sphagnum* peat (58%), sedge-moss peat (8%), sedge peat (19%) and few-flowered sedge (15%) (Mäkilä and Grundstöm 2008). The bottom soil below the peat layers consists of clay and sand derived from a former seabed and in the middle parts of the bog, gyttja (mud formed from decomposed peat) is found on top of the clay layer (Mäkilä and Grundström 2008). In the < 2 µm mineral fraction of the bottom soil layer clay minerals illite, kaolinite and clinochlore are found (present study) (Figure 2) and in the gyttja layer large amounts of diatoms have been observed

(*Bacillariophyta*) (unpublished data). Especially small Fragillaria species, common in sediments formed during the contraction of the Baltic Sea between the Limnea Sea and Litorina Sea –phases, have been identified (Figure 3).

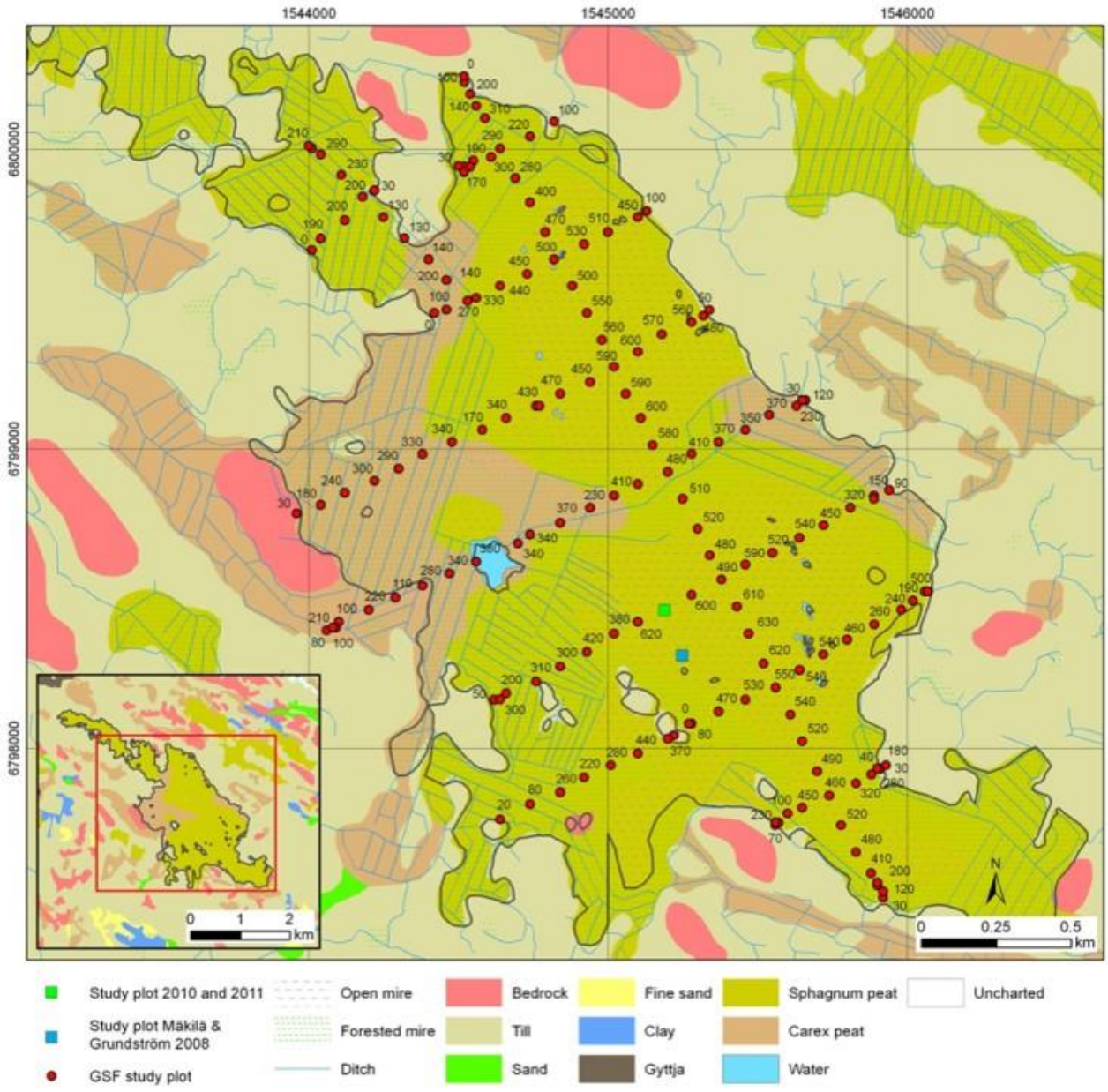


Figure 1. Map of Lastensuo bog. The samples used in present study have been taken from the study plot 2010 and 2011 marked with a green square in the middle part of the bog. The red points are study plots of Geological survey of Finland. (Map by J. Helin Posiva Oy).

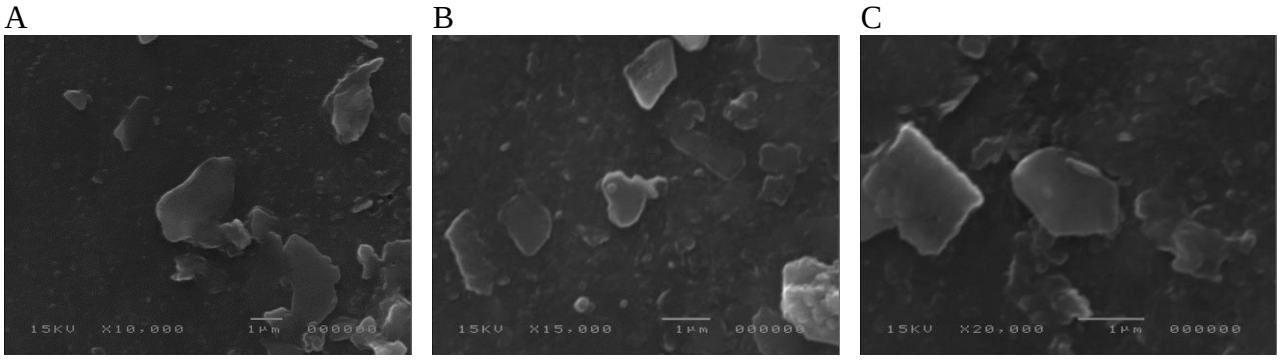


Figure 2. Electron micrographs of clinochlore (A), kaolinite (B) and illite (C) found in the < 2µm fraction of the Lastensuo bog layers from 5.5 – 7.0 m.

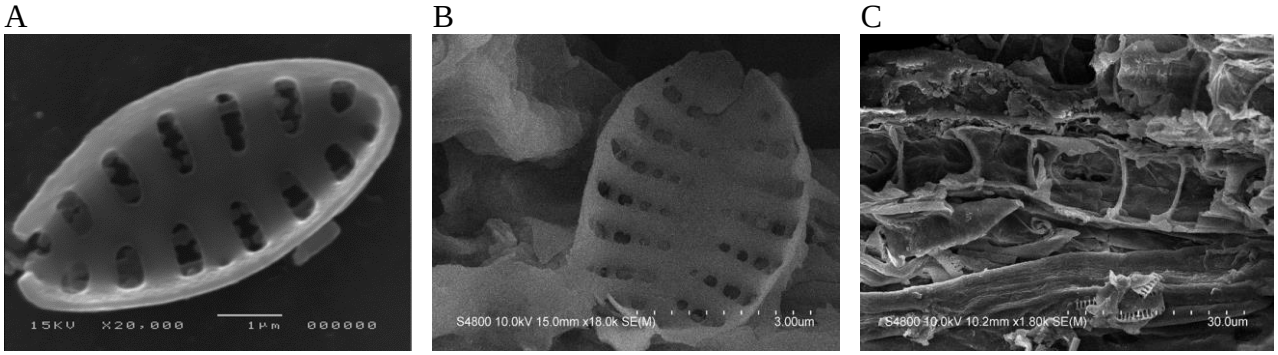


Figure 3. Electron micrographs from the Lastensuo bog gyttja layer (5.5 – 6.0 m). (A) A small Fragilaria -species (B) Diatom C) Undecayed plant tissue and diatoms.

Typically the microbial activity in peat profiles is highest in the acrotelm with higher oxygen concentration, but as a result of the respiratory activity of anaerobic microbes, microbial activity is also significant in the lower layers (catotelm) (Moore 1989). The majority of the microorganisms inhabiting northern, acidic bogs have not been isolated (Andersen et al. 2013), but since the developments in molecular biology technics (e.g. 16S rRNA sequencing) *Acidobacteria, α-proteobacteria, γ-proteobacteria, δ-proteobacteria, Verrucomicrobia, Actinobacteria, Chloroflexi, Planctomycetes, Sphirocaetes* and *Bacteroidetes* have been identified as dominant taxonomic groups in northern pristine and drained peat forest soils, acidic meso- and oligotrophic fens as well as in ombrotrophic bogs (Juottonen et al. 2005, Dedysh et al. 2006, Sun et al. 2014). The bacterial community of the Lastensuo bog profile has been determined using 16S rRNA gene based high throughput (HTP) amplicon sequencing (Tsitko et al. 2014). In the bog profile a total of 40 different bacterial phyla were identified, of which 13 phyla were found in all depths and covered 97 – 99 % of all sequence reads in each layer (Tsitko et al. 2014) (Figure 4). In the surface moss layer the majority of the bacterial community consisted of *Acidobacteria* and *Proteobacteria* with declining abundance in deeper layers. In the clay layer the relative amount of *Proteobacteria* was again increased. The abundances of *Cloroflexi, Verrucomicrobia* and *Spirochaeta* increased at greater depths and *Acidobacteria* were detected in all layers with maximum relative abundance at 2.5 – 4.0 m depth (Tsitko et al. 2014). The gyttja layer and bottom clay layer had greater bacterial diversity than the peat layers, and in addition to *Acidobacteria* they also contained *Verrucomicrobia, Chloroflexi,*

Bacteroidetes, *Spirochaeta* and *OP8* groups. The concentration of bacteria (number g^{-1} DW sample) was between 1-5 × 10^{10} in the surface moss, peat and gyttja layers and 5 × 10^{9} in the clay layer (Tsitko et al. 2014).

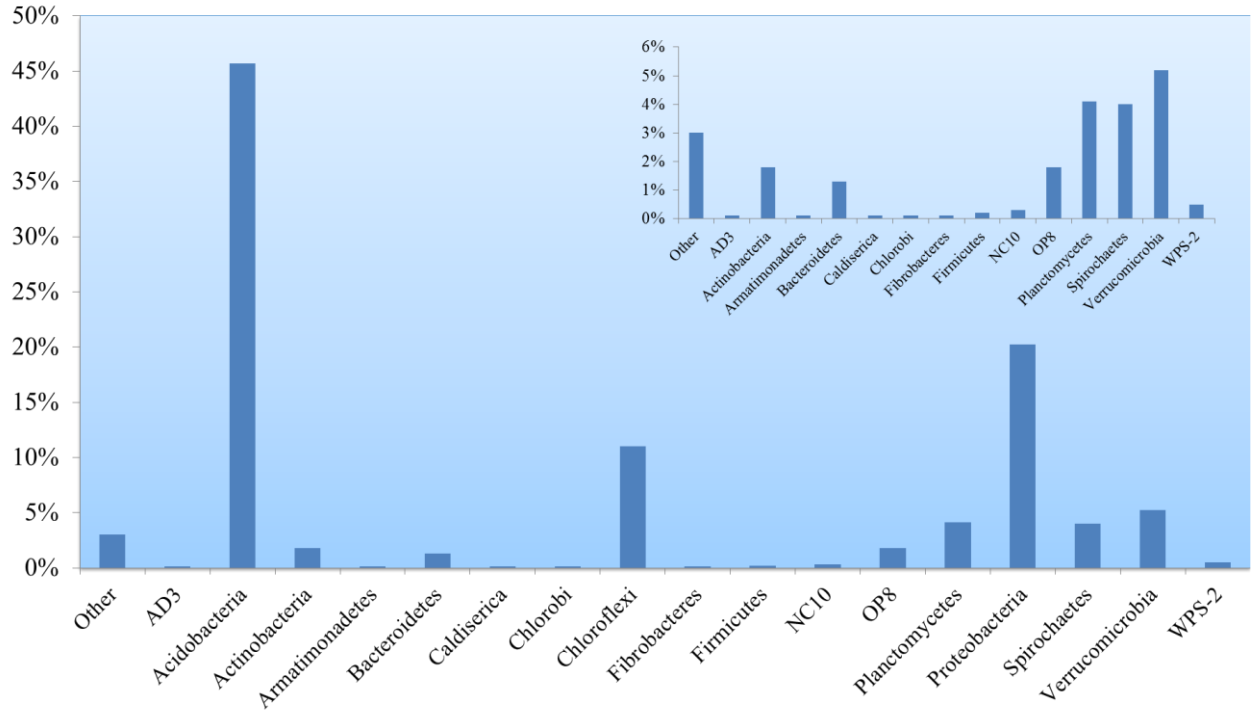


Figure 4. The relative abundance of all bacterial phyla identified in the Lastensuo bog (based on data from Tsitko et al. 2014).

The three most abundant phyla in Lastensuo bog profile were *Acidobacteria*, *Proteobacteria* and *Chloroflexi* (Figure 4). *Acidobacteria* is a large and diverse group, but only few species have been isolated from tundra soil and *Sphagnum* peat (Männistö et al. 2012, Pankratov and Dedysh 2010). Therefore their role in the elemental cycles is poorly known (Tsitko et al. 2014). In the depth profile of Lastensuo bog, *α-proteobacteria* were abundant from the surface moss layer to the depth of 4 m and the diversity among *α-proteobacteria* was high (Tsitko et al. 2014). These bacteria can inhabit various acidic environments and have been previously characterized in acidic peat bogs (e.g. Juottonen et al. 2005, Dedysh et al. 2006, Sun et al. 2014). *β-* and *γ-proteobacteria* were only detected in the moss and clay layer and *δ-proteobacteria* were the most abundant proteobacterial group in the 5.5 – 6.0 m depth of Lastensuo bog (Tsitko et al. 2014). δ- *proteobacteria* are important in the sulphur- and selenium cycle (e.g. *Desulfovibrio desulfuricans*) (Nelson et al. 1996). *Chloroflexi* contributed with approximately 10 % of the bacterial sequence reads in Lastensuo bog. Of these 97 – 100 % belonged to *Dehalococcoidetes*, of which all so far isolated strains are anaerobic obligate organohalide-respiring bacteria that use halogenated hydrocarbons as terminal electron acceptors (Hug et al. 2013). In organohalide-respiration halogen–carbon bond is broken and the halogen atom is liberated as a halide (Hug et al .2013).

4. The abiotic retardation of anionic and cationic radionuclides in the biosphere

The migration and retardation of both anionic and cationic radionuclides in the biosphere is affected by the surfaces of organic matter and minerals. In addition various biosorption, bioprecipitation and bioaccumulation processes are possible (described more detailed in section 5). Different retardation mechanisms including adsorption, incorporation (i.e. mineralization) and precipitation are expected in the surface biosphere and these mechanisms are affected by various environmental factors including pH of the soil solution, redox potential, organic matter content, mineral properties and microorganisms (e.g. Sheppard et al. 1995, Ashworth et al. 2003).

4.1. Mineral surfaces

Sorption mechanisms in mineral surfaces include outer- and inner-sphere complexation on hydroxyl groups (Figure 5) and sorption on interlayer and frayed edge sites (FES) of clay minerals (section 5.3) (Figure 11). These mechanisms occur between the charged surfaces of minerals and hydrated or partially hydrated ions. As the substance is hydrated, water molecules are attached to a charged atom forming a hydration shell. The water molecules in the hydration shell are orientated in the way resulting in a net charge of the same sing outside the shell as that of the ion in the centre. In outer-sphere complexation a hydrated cation or anion is attached to the charged surface through physical adsorption involving electrostatic interactions (i.e. dipole-dipole, ion-dipole, van der Waals interactions) (Atkins and de Paula 2002, Sparks 2003, Sposito 2008). In the inner-sphere – complexation the ion loses parts of its hydration sphere in order to enable the formation of a chemical bond between the sorbing ion and surface (Stumm 1992). From these mechanisms the strength of the inner-sphere complexation is considered higher, as the substances sorbed by outer-sphere complexation are readily desorbed if the ionic strength or pH of the solution is changed. Precipitation is possible when the solubility constant of respective substance is exceeded. In the present study the relevant precipitation processes are the enzymatically driven precipitation associated to the microbial removal of selenite from the solution, described more detailed in section 5.2.

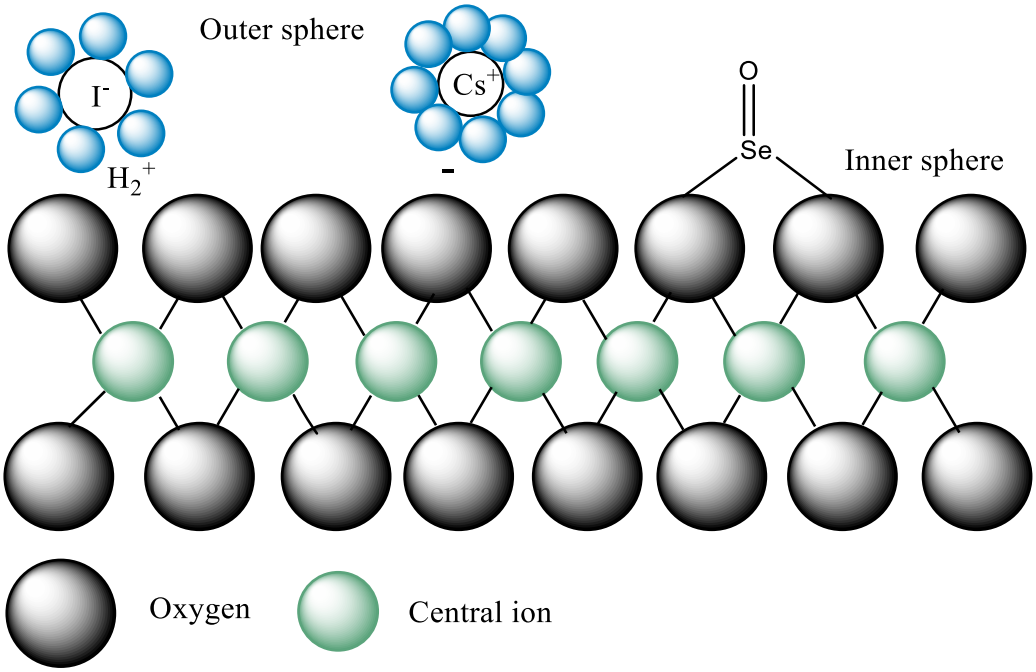


Figure 5. The inner and outer sphere complexation mechanisms of I^-, SeO_3^- and Cs^+.

Due to the replacements in clay mineral framework, such as Si^{4+} for Al^{3+}, clay minerals are characterized by permanent negative charge (Koch-Steindl and Pröhl 2001). This structural charge is stabilized by exchangeable cations adsorbed in the interlayer spaces and on the basal planes of clay minerals. In addition clay minerals also carry a variable charge in their terminal hydroxyl groups, which may be positive or negative, depending on the protonation of hydroxyl groups (Koch-Steindl and Pröhl 2001). In addition to clays important functional surface groups are also found in other silicate and oxide minerals, in which the most abundant functional surface groups are the hydroxyl groups associated with mineral-forming metals, such as silicon (-SiOH), aluminium (-AlOH), iron (-FeOH) and magnesium (-MgOH). The protonation of the amphoteric M-OH groups (where M is a metal atom in the bulk mineral) can be described as (Equation 1) (O'Day 1999):

$$\overbrace{M-OH_2^+}^{pH<pH_{pzc}} \overset{H^+}{\leftrightarrow} M-OH \overset{H^+}{\leftrightarrow} \overbrace{M-O^-}^{pH>pH_{pzc}} \quad (1)$$

Due to their increasing positive charge, these groups adsorb anions at pH values below the zero point of charge (pH_{pzc}) and vice versa for cations the sorption is increased above the zero point of charge. Large simple anions, such as I^-, and cations with small charge and/or large size, such as Cs^+, typically sorb on the charged groups by outer-sphere complexation (Equations 2 and 3, Figure 5) while oxoanions, such as IO_3^-, sorb by ligand exchange (inner-sphere complexation) (Equation 4) to form M-IO_3.

$$M - OH_2^+ + I^- \leftrightarrow M - OH_2^+ I^- \quad (2)$$

$$M - OH^- + Cs^+ \leftrightarrow M - OH^- Cs^+ \quad (3)$$

$$M - OH_2^+ + IO_3^- \leftrightarrow M - IO_3 + H_2O \quad (4)$$

The acidity of the mineral-forming metal affects the pH range in which the mineral is able to take up cations and anions. The silanol groups (Si-OH) remain unprotonated over the entire environmentally relevant pH region and are therefore not participated into the anion sorption. The most significant hydroxyl groups contributing to the anion sorption are those associated with iron and aluminium. These groups remain protonated up to a pH of about 8. Iron hydroxides are most important for the anion sorption at pH values below 5, while at a pH from 5 to 7, aluminium hydroxides become dominant (Whitehead 1974, Um et al. 2004).

4.2. Soil organic matter

The soil organic matter (SOM) is particularly diverse and consists of non-humic and humic substances of which non-humic substances include among others carbohydrates, proteins, lipids (fats, oils, resins, waxes) and lignin (Sparks 2003). The humic substances include humic acids (HA), fulvic acids (FA) and humin. The humic substances are a heterogenic group of large and complicated molecules and their classification is based on their solubility; FA is soluble in acidic and alkaline solutions, HA in alkaline solutions and humins are insoluble both in acidic and alkaline solutions. Surface functional groups of the humic substances are in a significant role in the adsorption processes of organic matter (Sparks, 2003) and the most important groups include acidic carboxylic groups (R-COOH), alcoholic and phenolic –OH groups and amino groups (R-NH_2) (Sparks 2003, Paasonen-Kivekäs et al. 2009, Tan 2003). Under alkaline conditions the carboxylic groups deprotonate, resulting in negative charge enabling electrostatic interactions between cations and negatively charged groups. The phenolic OH-groups are weakly acidic, but it is questionable if the pH values below the pH_{zpc} of phenolic-OH groups can induce the protonation of these groups and hence a positive charge (Tan, 2003). Instead the protonation of amino groups is possible in acidic conditions enabling the sorption of anions through electrostatic interactions (Equation 5):

$$R - NH_2 \overset{H^+}{\leftrightarrow} R - NH_3^+ \quad (5)$$

Other mechanisms associated in the removal of iodide and selenite from the solution in the presence of organic matter are discussed in sections 5.1. and 5.2.

5. The chemistry and microbial effect on I^-, SeO_3^{2-} and Cs^+ behaviour in the environment

5.1. Environmental chemistry of I^- and effects of microbes on its retardation

Because of the essential role of iodine in the thyroid hormones thyroxine (T4, tetraiodothyronine) and triiodothyronine (T3), iodine is an important trace element for humans and animals (De La Vieja et al. 2000, Eskandari et al. 1997). Iodine bioaccumulates in humans especially in the thyroid gland, through the food chain or inhalation (Xu et al. 2011a). In a recent nuclear deposition ^{131}I causes the most significant radiological hazard during the first months after the deposition. From the radioecological point of view, ^{129}I is among the most important radionuclides in the long-term safety assessments of SNF (Helin et al. 2010). ^{129}I is produced in nuclear reactors as a fission product of ^{235}U and the yield of ^{129}I per fission is high (Nichols et al. 2008). High fission yield together with the long half-life of 15.7 My results in a large inventory of ^{129}I in spent nuclear fuel. Small amounts of ^{129}I is also produced naturally from isotopes of Xe by cosmic radiation in the atmosphere and by spontaneous fission of ^{235}U in the earth's crust (Edwards 1962).

Stable iodine has an average concentration of 0.3 ppm in the earth's crust (Fuge and Johnson 1986) and in soils the concentrations are generally greater. For soils iodine concentrations between 3 – 30 ppm have been reported (Yuita 1992, McGrath and Fleming 1988). Several factors affect the migration and sorption of iodine in the geosphere, including its chemical speciation, organic matter content, mineral properties, redox potential, pH and microorganisms (Assemi and Erten 1994, Evans and Hammand 1995, Sheppard et al. 1995, Ashworth et al. 2003, Ashworth and Shaw, 2006, Li et al. 2012). Iodine is predominantly retained in SOM (Bostock et al. 2003, Yamaguchi 2010, Xu et al. 2011a, Li et al. 2012, Xu 2013), and microorganisms have been reported to affect the sorption of iodine in several studies (e.g. Bunzl and Schimmack 1988, Muramatsu et al. 1990, Assemi and Erten 1994, Evans and Hammad 1995, Yamaguchi et al. 2010, Li et al. 2011, Li et al. 2012, Xu et al. 2013), although the actual mechanism has not been discussed until recently (e.g. Li et al. 2011, Li et al. 2012, Xu et al. 2013).

Major iodine species found in the environment include iodide (I^-), iodate (IO_3^-), molecular iodine (I_2) and organo-iodine (Muramatsu et al. 1990, Muramatsu and Yoshida 1999, Li et al. 2012, Xu et al. 2013, Kaplan et al. 2014). In the environments with high organic matter content, organo-iodine forms a major proportion of the total iodine (Xu et al. 2013). In anoxic, waterlogged environments with low organic matter content, iodine typically appears as I^- and in oxic environments as IO_3^- (Yuita 1992, Ashworth et al. 2003, Ashworth and Shaw 2006, Li et al. 2012) (Figure 6).

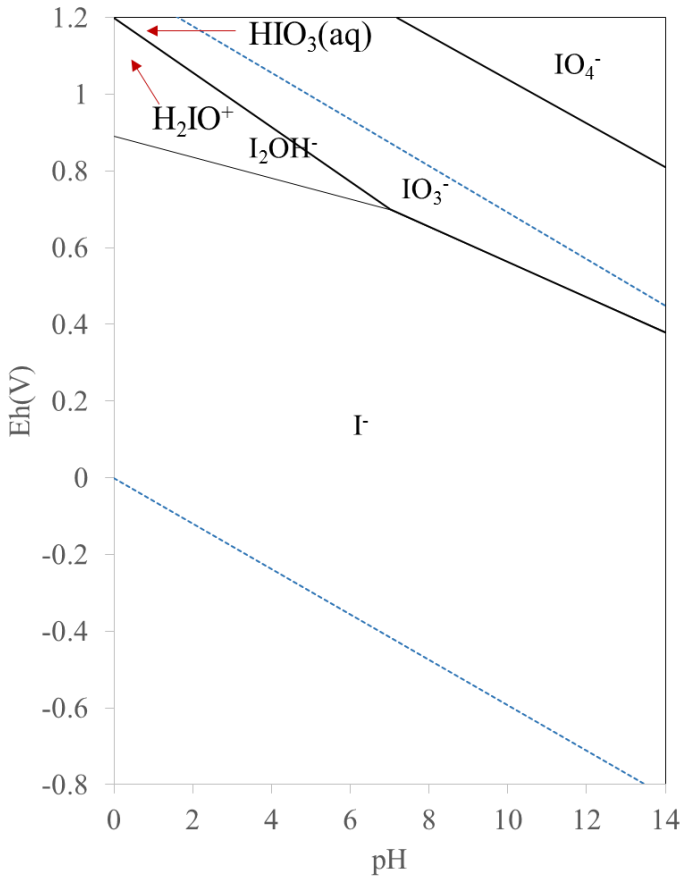


Figure 6. Speciation of iodine in water as a function of pH and Eh. The blue lines represent the stability limits of water. Upper line represents the pH-Eh line in which O_2 is formed and lower line in which H_2 is formed. (Adapted from Takeno 2005).

Because of its thermodynamically unfavourable oxidation via single-step electron transfer without a strong oxidant, I^- is assumed stable over typical pH and Eh ranges (Li et al. 2012) (Figure 6). Abiotic oxidants such as MnO_2 and Fe_2O_3 are known to oxidize I^-, but the significance of these reactions is limited under environments with pH values below 5 (Xu et al. 2011b). In addition humic substances can act as oxidizing agents for I^- (Keller et al. 2009), but in SOM I^- oxidation is linked to the extracellular enzyme activity of soil microbiota (Bunzl and Schimmack 1988, Evans and Hammad 1995, Koch-Steindl and Pröhl 2001, Li et al. 2012, Muramatsu et al. 1990, Sheppard and Hawkins 1995, Yoshida et al. 1998).

In organic soils, I^- oxidation produces numerous highly reactive intermediates, and it has been shown in multiple studies that I^- is oxidized into an intermediate such as I_2 or hypoiodous acid (HIO) prior to its interaction with soil organic matter through iodination (Warner et al. 2000, Reiller et al. 2006, Schlegel et al. 2006, Li et al. 2012, Xu et al. 2013, Xu et al. 2011, Yamaguchi et al. 2010). The oxidation of halides, including iodide, is mediated by microbial peroxidases including haloperoxidases. Haloperoxidases are widely found in the environment and catalyze the H_2O_2 oxidation of I^- into electrophilic I_2 or HIO species (Lin and Chao 2009) (Equations 6 – 7):

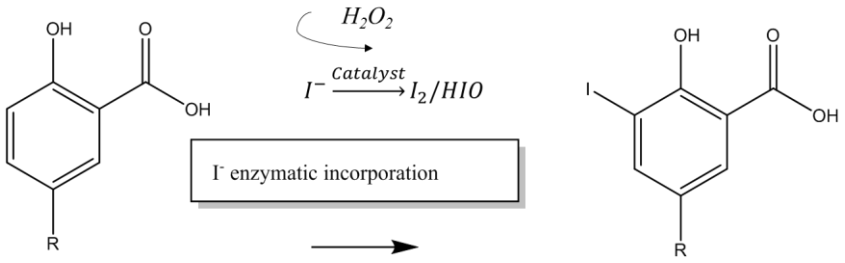
$$2\,I^-(aq) + 2H^+(aq) + H_2O_2(aq) \rightarrow I_2(s) + 2H_2O(l) \quad (6)$$

$$I^- + H^+ + H_2O_2 \rightarrow HIO + H_2O \quad (7)$$

In soils H_2O_2 is naturally produced by UV radiation and from metabolic processes of both fungi and bacteria under aerobic conditions (Xu et al. 2013, Li et al. 2012). I_2 is formed especially in oxidizing, acidic environments such as bogs (Li et al. 2012, Xu et al. 2013, Kaplan et al. 2014), and in aqueous solutions I_2 is readily hydrolysed to HIO by the reaction (Equation 8) (Nagy et al. 2003):

$$I_2 + H_2O \leftrightarrow HIO + I^- + H^+ \quad (8)$$

As the dissociation constant (pK_a) of HIO is 10.4 (Bichsel and von Gunten 2000), its undissociated form, IO^-, is not relevant in acidic and neutral environments. It is expected that I_2/HIO reacts with organic matter to form organo-iodine compounds (Yamaguchi et al. 2010, Xu et al. 2011a, Li et al. 2012, Seki et al. 2013, Xu et al. 2013) via covalent C-I bonds (Xu et al. 2011). Recently, it has been demonstrated using spectroscopic methods, that I^- is catalytically oxidized into reactive iodine species (e.g. I_2 or HIO) by peroxides and at the same time fulvic acid is oxidized by peroxides into more aliphatic and less aromatic compounds on which reactive iodine is bound to form new organo-iodine compounds (Xu et al. 2013) (Equation 9, Adapted from Xu et al. 2013):



(9)

In the mineral soils, important functional surface groups are found in silicate and oxide minerals. As stated above in section 4.1. the most abundant functional surface groups in these minerals are the hydroxyl groups associated with mineral-forming metals, such as silicon, aluminium, iron and magnesium. These amphoteric M-OH groups are protonated at low pH values and deprotonated at higher pH levels (Equation 1, section 4.1.). As described in section 4.1. I^- sorbs on the positively charged groups by outer-sphere complexation (Equation 2, Figure 5) while oxoanions, like IO_3^-, is sorbed by inner-sphere complexation (Equation 4, Figure 5) to form M-IO_3. Consequently, the

hydroxyl groups of the mineral surfaces favour IO_3^- over I^- as I^- sorbed by outer-sphere complexation is readily exchangeable.

Until lately, the uptake of iodine by living cells has been characterized only in few organisms; the mammalian thyroid gland (e.g. De La Vieja et al. 2000), other vertebrates (e.g. Eskandari et al 1997), and in marine algaea, such as *Laminaria* spp. (Küpper et al, 1998). The uptake mechanism has been described most comprehensively in the mammalian thyroid gland, in which iodine is taken up as I^- by an active transport process against the I^- concentration gradient using a sodium/potassium symporter (Na^+-K^+-ATPase) (De La Vieja et al. 2000). The biogeochemical cycling of iodine is known to be affected by microorganisms (e.g. Li et al. 2012, Xu et al. 2013, Yamaguchi et al. 2010, Li et al. 2011), but only a few studies on iodine uptake by bacteria have been published (e.g. Li et al. 2011, Amachi et al. 2007, Amachi et al. 2010). However, a hydrogen peroxide-dependent uptake of I^- by a marine *Flavobacteriaceae* bacterium strain C-21, in which I^- is oxidized to I_2 or HIO by haloperoxidase before incorporation into the bacterial cell, has been suggested by Amachi et al. (2007) (Figure 7). In this facilitated diffusion mechanism (a passive process in which molecules are transported across the cell membrane via special transport proteins), glucose oxidase is also present, oxidising glucose into gluconate and H_2O_2, needed in the further oxidation processes.

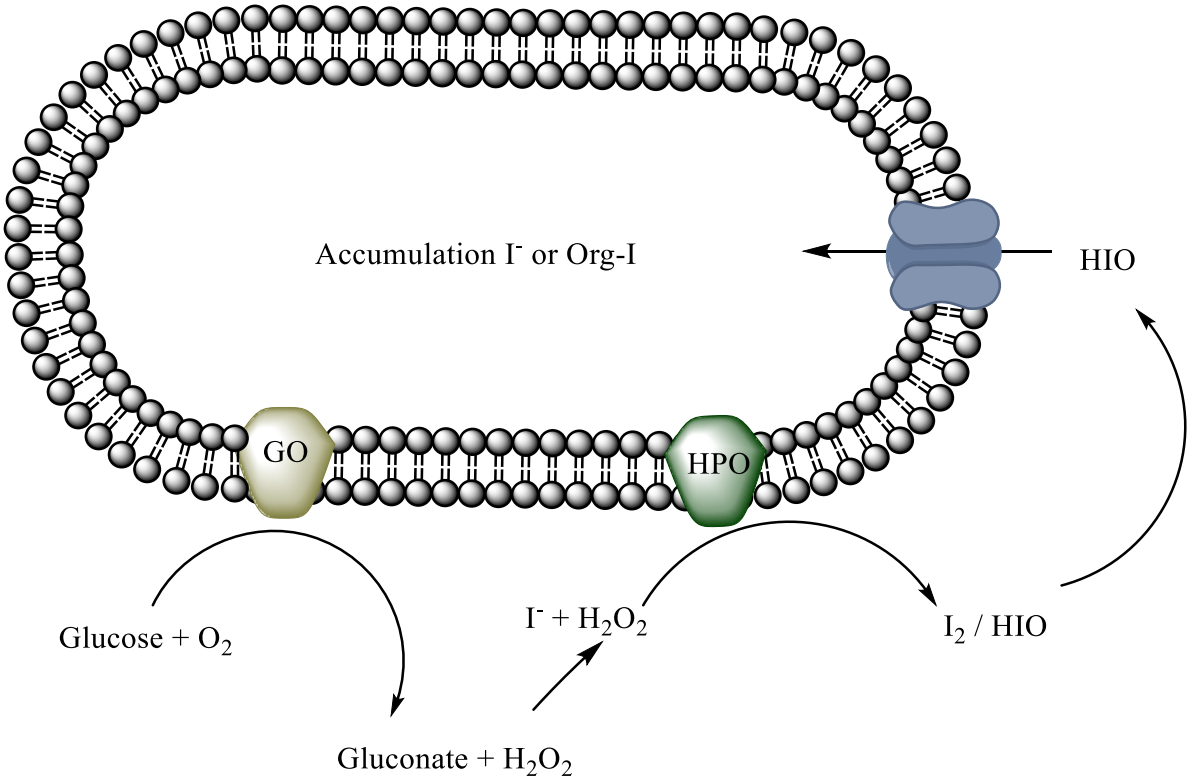


Figure 7. Schematic representation of possible mechanism of I^- uptake and accumulation in Flavobacteriaceae strain C-21 through facilitated diffusion suggested by Amachi et al. 2007. For clarity, the periplasmic space and outer membrane are not shown. GO = glucose oxidase, HPO = haloperoxidase. (Adapted from Amachi et al. 2007).

A study on the ability of anaerobic microorganisms to associate with iodine was also published by Amachi et al. (2010) and the results showed very limited adsorption or accumulation of iodine by anaerobic microorganisms. I^- accumulation in three aerobic bacterial strains from the subsurface sediments of Savannah River Site, FA-30, FA-2C-B, and FA-191, closely related to *Streptomyces/Kitasatospora* spp., *Bacillus mycoides*, and *Ralstonia/Cupriavidus* spp. has however been described (Li et al. 2011). The bacterial oxidation of iodine has been studied increasingly (e.g. Li et al. 2012, Gozlan 1968, Amachi et al. 2005, Li et al. 2014) since the late 1960s when Gozlan (1968) isolated an iodide-oxidizing bacterium from experimental seawater aquaria. Gozlan and Margolith (1973) named this bacterium *Pseudomonas iodooxidans* sp. nov., but the culture stock of this bacterium was lost and therefore the mechanism of I^- oxidation, as well as the prevalence of *P. iodooxidans* in the environment is unknown (Amachi et al. 2005, Li et al. 2014). Iodide-oxidizing bacteria have been isolated from iodide-rich natural gas brines and the isolates were phylogenetically divided into two groups within the α-proteobacteria (Amachi et al. 2005). One of the groups affiliated with the *Roseovarius* lineage and the other group represented a phylogenetically distinct group of previously characterized bacteria (Amachi et al. 2005). A H_2O_2-dependent I^- oxidation mechanism involving organic acids produced by the bacteria has been described in ten bacterial strains isolated from soil in the F-area of Savannah River Site (Li et al. 2012). These bacterial isolates belonged to *Actinobacteria, Bacteroidetes, Firmicutes* and *Proteobacteria* phyla (Li et al. 2012). In addition, a manganese-oxidizing marine bacterium *Roseobacter* sp. AzwK-3b was recently reported to produce superoxide facilitating the I^- oxidation (Li et al. 2014).

In addition to biosorption and accumulation of radionuclides by microbiota, microbially-generated organic acids can cause changes in pH and the redox conditions can be changed due to microbial oxidation/reduction reactions (Tamponnet et al. 2008, Joergensen and Emmerling 2006). These changes further affect the migration, sorption and geo- and biochemical circulation of radionuclides.

5.2. Environmental chemistry of SeO_3^{2-} and effects of microbes on its retardation

As the possible radiation doses for humans in the future, following the hypothetical releases from the deep bedrock repository of nuclear fuel is considered, ^{79}Se is classified as a high priority radionuclide in the long-term safety assessment calculations (Helin et al. 2010). ^{79}Se is a fission product and in addition it is formed by neutron activation from stable selenium by reaction ^{78}Se (n, γ) ^{79}Se and it has a long half-life of 1.13 My. In addition considerable amounts of stable selenium enter the environment via anthropogenic activities including coal combustion, mining, refining of sour crude oils and agricultural irrigation of seleniferous soils (Coppin et al. 2009, Sharmasarkar and Vance 2002, Manceau and Gallup 1997, Yasin et al. 2014, Souza et al. 1999). Even though selenium is an essential micronutrient for humans and animals, it becomes toxic with higher concentrations and is characterized by a narrow range between toxic and deficient doses (Terry et al. 2000, Barceloux 1999).

The behavior of selenium in the environment is influenced by several factors such as pH, chemical form, soil mineral composition, redox conditions, as well as micro-organisms (Nakamaru and

Altansuvd 2014, Sarret et al. 2005, Nelson et al. 1996, Oremland et al. 2004, Souza et al. 1999). In the environment selenium occurs with different oxidation states forming selenide (Se^{2-}), elemental Se (Se^0), selenite (SeO_3^{2-}), selenate (SeO_4^{2-}) and organic Se (Kausch et al. 2012, Pezzarossa et al. 1999). At high redox potential SeO_4^{2-} dominates and at intermediate redox conditions SeO_3^{2-} becomes more prevailing (Nakamaru and Altansuvd 2014, Pezzarossa 1999) (Figure 8). Se^{2-} and Se^0 are found typically in most reducing environments with low pH (Nakamaru and Altansuvd 2014, Pezzarossa 1999) and SeO_3^{2-} sorption in soils and minerals has been reported to decrease with increasing pH and competition with more adsorbing anions such as phosphate (PO_4^{3-}), arsenate (AsO_4^{3-}) or bicarbonate (HCO_3^-) (e.g. Lee et al. 2011, Su and Suarez 2000, Balistieri and Chao 1987, Missana et al. 2009).

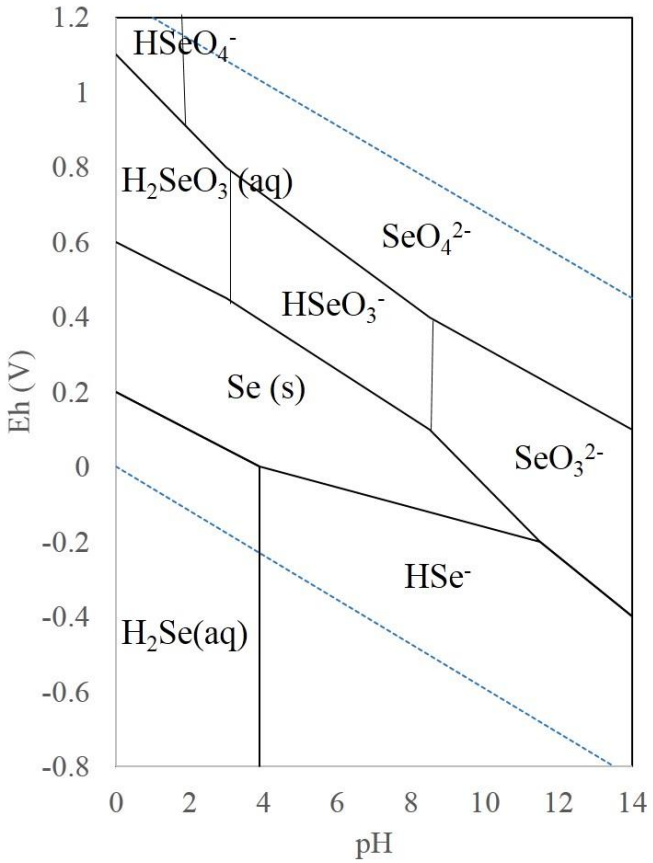


Figure 8. Speciation of selenium in water as a function of pH and Eh. The blue lines represent the stability limits of water. Upper line represents the pH-Eh line in which O_2 is formed and lower line in which H_2 is formed. (Adapted from Takeno 2005).

In mineral soils the solubility of selenium is affected by adsorption on oxy-hydroxides of aluminum (Al), iron (Fe) and manganese (Mn). SeO_3^2 forms inner-sphere bidentate surface complexes with hematite (Catalano et al. 2006, Balistieri and Chao 1990), amorphous $Fe(OH)_3$ (Balistieri and Chao 1990, Su and Suarez 2000) and goethite (α-FeOOH) (Su and Suarez 2000) (Figure 5). When comparing the sorption of the two oxyanions, SeO_3^2 is known to adsorb more strongly on amorphous iron oxyhydroxide and manganese dioxide compared to SeO_4^2, (Balistieri and Chao 1990). SeO_3^{2-}

adsorption on amorphous iron oxyhydroxides and manganese dioxide has been reported to increase with decreasing pH (Balistieri et al. 1990). In suboxic sediments and soils containing Fe(II,III) oxides, a slow abiotic reduction (>1 month) of SeO_4^{2-} to Se^0 has been observed, but only under reducing conditions at pH below 7 (Charlet et al. 2007). In addition under anoxic conditions an abiotic reaction of SeO_3^{2-} with sulfide (formed biotically) forming insoluble Se^0 is possible (Equation 10) (Pettine et al. 2012):

$$SeO_3^{2-} + 2\,HS^- + 4\,H^+ \rightarrow Se^0 + 2\,S^0 + 3\,H_2O \qquad (10)$$

In organic wetland soils the microbial reduction of SeO_4^{2-} and SeO_3^{2-}into insoluble elemental Se^0 is an important process which greatly affects the environmental distribution and biological effects of selenium (Nakamaru and Altansuvd 2014, Li et al. 2014). Both Archaea and Bacteria are known to use SeO_4^{2-} and SeO_3^{2} as terminal electron acceptors and to reduce soluble SeO_4^{2-} and SeO_3^{2-} to insoluble Se^0 under anoxic conditions (e.g. Fujita et al. 1997, Sarret et al. 2005, Li et al. 2014, Huber et al. 2000) primarily via microbial dissimilatory reduction involving enzymes with molybdenum co-factors and a number of organic substrates (e.g. acetate, lactate, pyruvate, glycerol and ethanol) or hydrogen (Stolz and Oremland 1999). Dissimilatory reduction produces electrochemical gradients, which provide the chemical energy required for the growth. In addition, under aerobic or microaerophilic conditions, SeO_3^{2-} is reduced to Se^0 by various bacterial strains, either through detoxification mechanisms or redox homeostasis (phototrophic bacteria) (Tejo et al. 2009). Detoxification of SeO_3^{2-} to Se^0 can take place using various mechanisms including Painter-type reactions, the thioredoxin reductase system, and sulfide- and siderophoromediated reduction (Nancharaiah and Lens 2015) (Figure 9). A further microbiologically mediated reduction of Se^0 into soluble Se^{2-} is possible and Se^{2-} can react with metal ions to form insoluble metal selenides. Selenium species are also found in organoselenium compounds, like selenols and selenyl halides, and methylated selenium species. Bacteria able to oxidize Se^0 and Se^{2-} back to SeO_3^{2-} (and SeO_4^{2-}) are also known (Nancharaiah and Lens 2015).

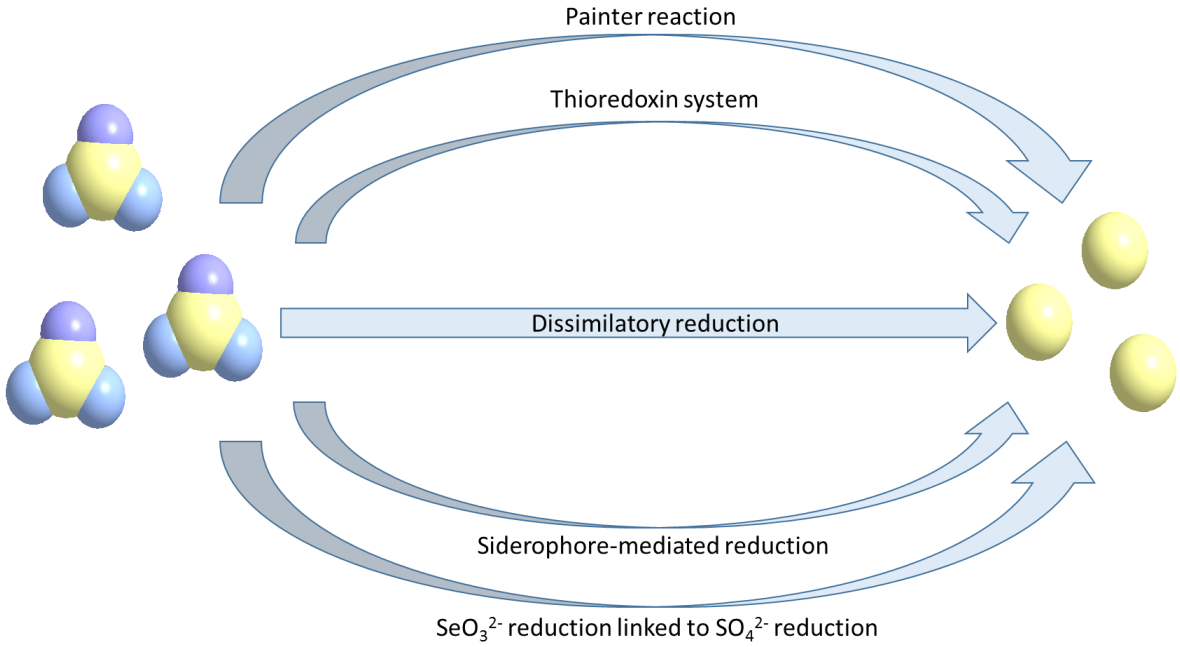


Figure 9. Reduction mechanisms of SeO_3^{2-} found in microorganisms. (Adapted from Nancharaiah and Lens 2015).

In the Painter-type reactions (Figure 9) SeO_3^{2-} reacts with thiols (HS-groups) forming selenotrisulphide (RS-Se-SR, e.g. glutathione selenotrisulfide GS-Se-SG) (Equation 11) (Haratake et al. 2005, Nancharaiah and Lens 2015):

$$6\,GSH + 3\,SeO_3^{2-} \rightarrow 3\,GS - Se - SG + 3\,O_2 + 3H_2O \qquad (11)$$

In aerobic and in some anaerobic bacteria GS-Se-SG is further reduced to GS-Se^- using glutathione reductases (Nancharaiah and Lens 2015, Swearingen et al. 2006). GS-Se^- is unstable and is converted to Se^0 through hydrolysis (Equation 12):

$$GSSe^- + H^+ \rightarrow GSH + Se^0 \qquad (12)$$

In the thioredoxin system reduced thioredoxin (a redox protein acting as antioxidant) and thioredoxin reductase found in *Escheria Coli* are hypothesized to be involved in the SeO_3^{2-} reduction into Se^0 (Nancharaiah and Lens 2015) and in *Pseudomonas stutzeri* KC a siderophore mediated reduction using iron siderophore (small iron chelating agent, 2,6-Pyridinedicarbothioic acid, PDTC) has been described (Zawadzka et al. 2006).

In dissimilatory reduction the reduction of metals is coupled to the oxidation of simple organic acids and alcohols or aromatic compounds in order to conserve energy (Lovley 1993). For example for SeO_3^{2-} reduction using lactate as electron donor the following reaction can be written as follows (Equation 13) (Lovley 1993, Nancharaiah and Lens 2015):

$$C_2H_4OHCOO^- + SeO_3^{2-} + H^+ \rightarrow CH_3COO^- + Se^0 + HCO_3^- + H_2O \quad (13)$$

In addition to above described mechanisms, some microorganisms (e.g. *Clostridium pasteurianum, Rhizobium sullae*) use reductases, e.g. sulfite reductase, fumarate reductase FccA, OYE enzyme (Old Yellow Enzyme, NAPDH oxidoreductase) and nitrite reductases in the SeO_3^{2-} reduction (Harrison et al. 1984, Basaglia et al. 2007, DeMoll-Decker and Macy 1993, Li et al. 2014, Hunter 2014).

Selenium respiring bacteria are found in a wide range of environments and they are dispersed throughout the bacterial domain (Stolz and Oremland 1999, Oremland et al. 2004, Li et al. 2014). Both intracellular and extracellular selenium nanogranules have been found in phylogenetically and physiologically distinct bacteria like *Chromatium vinosum, Desulfovibrio desulfuricans, Sulfospirillum barnesii, Bacillus selenitireducens, Selenihalanaerobacter shriftii, Shewanella oneidensis* MR-1, *Paenibacillus selenitireducens* sp. nov. and *Ralstonia metallidurans* CH34 (e.g. Nelson et al. 1996, Oremland et al. 2004, Li et al. 2014A). The genesis of intracellular and extracellular Se^0 nanospheres is still partly unsolved, particularly concerning the secretion of intracellularly synthesized Se^0 and it seems possible that internal and external Se^0 nanospheres are formed by different and independent mechanisms (Nancharaiah and Lens 2015). It has been suggested that the uptake of SeO_3^{2-} in *E. coli*. employs the sulphate ABC transporter complex composed of two CysA ATP-binding proteins, two transmembrane proteins (CysT and CysW) and a periplasmic sulphate binding protein (CysP) (Rosen and Liu 2009, Turner et al. 1998) (Figure 10). In *S. oneidensis* MR-1 Se^0 is formed in the periplasmic compartment (Nancharaiah and Lens 2015) and in *R. metallidurans* CH34 organoselenium compounds of form R-Se-R are also formed (Sarret et al. 2005). It is likely that an alternative, still unidentified carrier also exists for SeO_3^2, because the repression of SO_4^{3-} permease expression does not completely inhibit the SeO_3^{2-} uptake in *E. coli* (Turner et al. 1998). The transport from the cell has been proposed via cell lysis (*D. desulfuricans*) and vesicular secretion (*Rhodospirillum rubrum*) (Tomei et al 1995, Kessi et al. 1999). Even though in addition to reduction of SeO_4^{2-}, the reduction of SeO_3^{2-} into elemental selenium has been shown to be an environmentally significant process, only a few SeO_3^2-respiring bacteria have been isolated (Stolz et al. 2006).

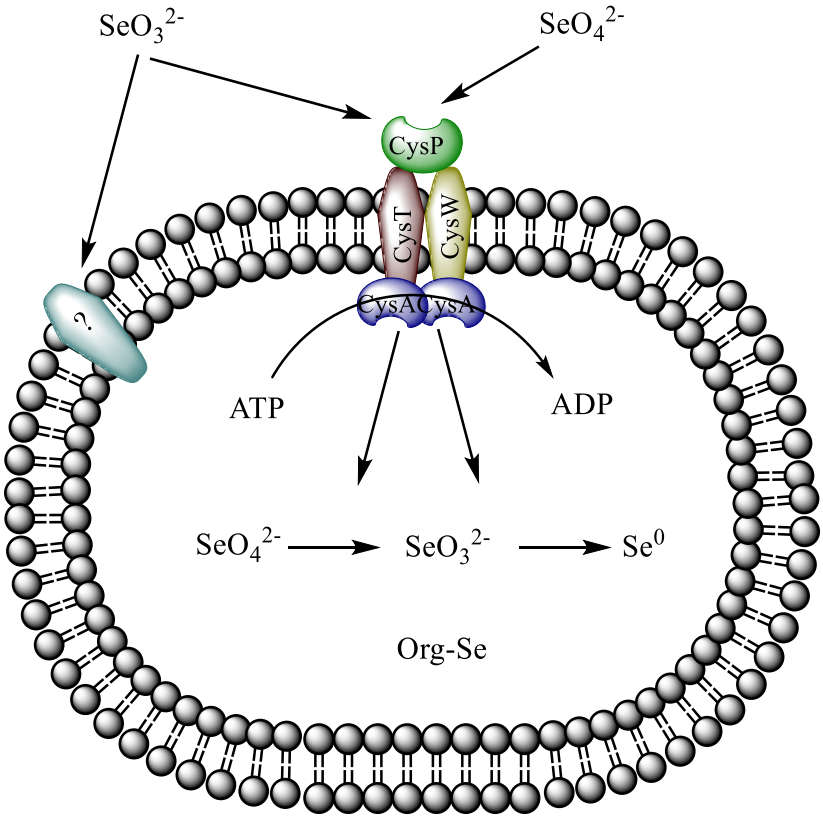


Figure 10. The schematic representation of selenate and selenite uptake in E. coli. For clarity, the periplasmic space and outer membrane are not shown. (Adapted from Rosen and Liu 2009, and Turner et al. 1998).

Although only a few studies exist on the specific interactions of selenium with organic matter (e.g. Bruggerman et al. 2007, Kamei-Ishikawa et al. 2008), organic-bound selenium is expected to have an important role in the biogeochemistry of wetland soils (Nakamaru et al. 2014). The actual mechanism of selenium association with organic matter is still not explained, but organic fractions have been shown to be the major carriers of selenium (Coppin et al. 2009). It has been suggested that selenium sorption on organic particles could only be indirect, mainly resulting from association with Fe oxides or clay minerals residing either on the organic matter surface or fixed within its matrix (Coppin et al. 2009) and is linked to the microbial reduction, which in turn is affected by the local chemical conditions of the soil (Kausch et al. 2012). In addition, nitrogen-containing groups ($-NH_2$) found in organic matter are protonated under acidic conditions (Equation 5) and SeO_3^{2-} sorption onto the resulting positively charged groups through electrostatic interactions is possible.

5.3. Cs^+ sorption mechanisms and biosorption

^{135}Cs is a fission product and classified among the important radionuclides in long-term safety assessments, due to its long half-life of 2.3 My and large inventory in SNF. In the biosphere assessment modelling the possible annual landscape dose caused by ^{135}Cs starts to increase approximately 2000 years after the disposal and will be in its highest (appr. 10^{-13}Sv) about 10 000 years after the disposal (Hjerpe and Broed 2010). As an alkali metal, cesium is potentially highly soluble and it occurs in the SNF essentially in the instant release fraction (IRF), which represents the fraction of safety-relevant radionuclides that will be released from the SNF at a faster dissolution rate than the matrix. In soils stable cesium is fairly rare and typical concentrations of approximately 5 mg/kg have been reported (Sparks et al. 2003). In minerals, cesium is found primarily in micas and K-feldspar, in which cesium partly substitutes potassium and in aqueous solutions cesium exists as free Cs^+ ions (Lieser and Steinkopff 1989). Changes in redox and pH conditions do not affect the speciation of cesium (Lieser and Steinkopff 1989).

5.3.1. Sorption mechanisms of Cs^+ on surfaces of minerals

Cs^+ is readily sorbed on the surfaces of soil components and in water on the surfaces of colloids and suspended particles (Chang et al. 1993, Zhuang et al. 2003). Typically Cs^+ is sorbed by outer-sphere complexation (ion exchange) (Figure 5), but in clay and mica minerals cesium also forms inner-sphere complexes (Bostick et al. 2002) (Figure 11). Outer-sphere complexes are formed between hydrated cesium and negatively charged surfaces of iron (Fe), manganese (Mn) or aluminium (Al) oxides, as well as on the functional groups of organic matter. However, the selectivity of cesium sorption by outer-sphere complexation on Fe, Mn and Al oxides as well as organic matter is considerably lower than on clay and mica minerals (e.g. Saengkul et al. 2013, Chang et al. 1993, Staunton et al. 2002). In inner-sphere complexes, partially or fully dehydrated Cs^+ coordinates directly to the siloxane groups of clay minerals within the interlayer or at FES of the mineral (Bostick et al. 2002, Lieser and Steinkopff 1989) and on these sites sorption is practically irreversible (Gutierrez and Fuentes 1996).

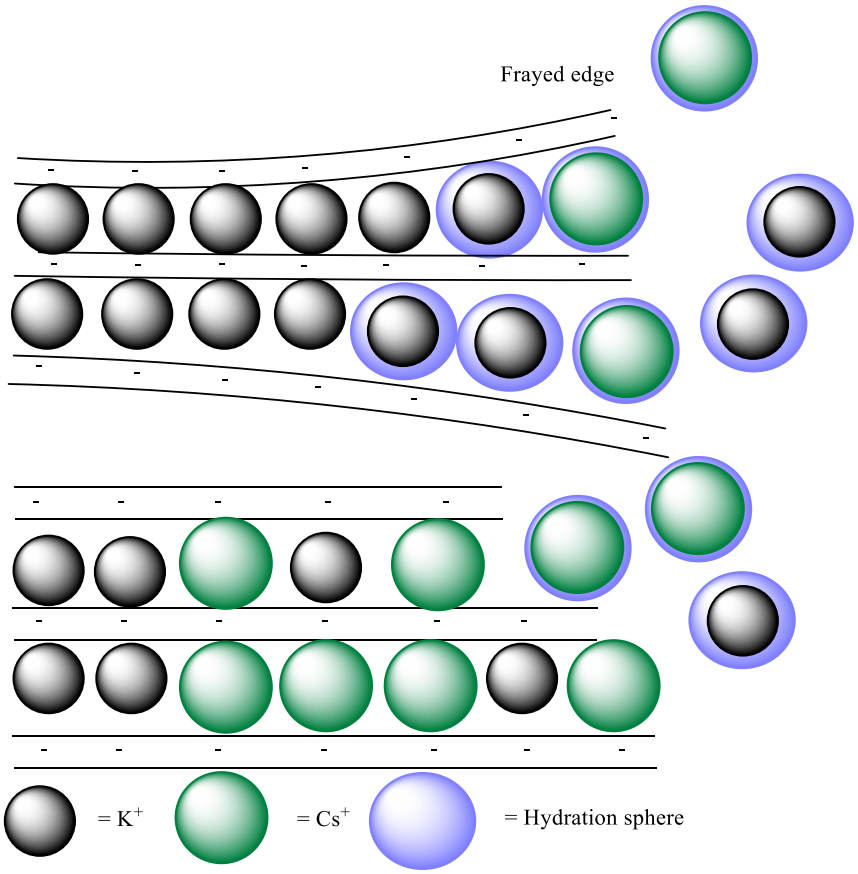


Figure 11. Cesium sorption on frayed edge sites (FES) of clay minerals (illite) (Adapted from Nakao et al. 2008).

5.3.2. Sorption mechanisms of Cs^+ on SOM

In SOM, Cs is sorbed on the deprotonated functional groups, of which the most important groups associated with cation adsorption include weakly acidic carboxylic groups (R-COOH) and alcoholic and phenolic –OH groups (Sparks 2003, Paasonen-Kivekäs et al. 2009, Tan 2003). Under alkaline conditions these amphoteric groups deprotonate, resulting in negative charge enabling electrostatic interactions between Cs^+ and negatively charged groups (Equation 14):

$$R - COOH \xrightarrow{pH>pH_{pzc}} R - COO^- Cs^+ \qquad pK_a\ 3 - 4 \qquad (14)$$

In the acidic bog environment, examined in the present study, limited sorption of Cs^+ on the organic matter was expected under *in situ* pH conditions, as majority of the functional groups are expected protonated under these conditions.

5.3.3. Sorption mechanisms of Cs^+ on bacteria

Bacterial surfaces have several extracellular functional groups, on which adsorption, similarly to mineralogical processes, is possible (Anderson et al., 2011). These groups include carboxyl (R-COOH), phosphate ($R\text{-}PO_4H_2$) and hydroxyl (R-OH) sites, which deprotonate as pH increases, typically increasing the biosorption capacity of bacteria in more alkaline conditions. Besides biosorption, also other mechanisms including intracellular accumulation and precipitation are found among bacteria (Anderson et al., 2011). In microbes the uptake of alkali metals (including Cs^+) is facilitated by monovalent cation transport systems located on the plasma membrane (Avery 1995) These mechanisms can differ widely in their specificity for each alkali metal and therefore substantial variety in the ability to accumulate Cs^+ is found among microorganisms (Avery 1995). In certain microorganisms Cs^+ seems to have an equal or greater affinity for transport than K^+ and microbial Cs^+ accumulation is commonly accompanied by a stoichiometric exchange for intracellular K^+ (Avery 1995). Only limited data is available on the biosorption of cesium by bacteria. For the sulphate-reducing bacteria isolated from a mine dump site a maximum uptake value of 238 mg/g bacterial biomass (dry weight) for Cs^+ has been demonstrated under anaerobic conditions (Ngwenya and Chirwa 2010). In terms of K_d values, Sasaki et al. (2002) reported biosorption K_d values below 100 L/kg after one to 120 days of incubation for cesium using anaerobic iron- and sulphate-reducing bacterial mixtures originally used for the treatment of waste water from pulp and paper plant. Bacteria isolated from deep groundwater, including among others *Pseudomonas fluorescens* C50-1, *P. stutzeri* 116-1-1, *P. putida* C49-2, *Sphingomonas panii* 116-2-2 and *Acinetobacter johnsonii* C61-2, have been reported not to sorb cesium (Luk'yanova et al. 2008), while the exopolymers (PFC02) produced from *P. fluorescens* C-2 were able to remove Cs from an aqueous solution (Mao et al. 2011). Mao et al. (2011) concluded that in exopolymers of *P. fluorescens* C-2 the potential functional groups responsible for adsorption were hydroxyl (R-OH), carboxyl (R-COOH), carbonyl (C=O) and sulfonate groups ($R\text{-}SO_2O^-$) (Mao et al. 2011).

6. Aims of the study

The aim of this study was to obtain knowledge about the sorption and retention of I^-, SeO_3^{2-} and Cs^+ in the moss, peat, gyttja and clay layers of the nutrient-poor boreal bog and the biotic and abiotic factors affecting their behaviour. These factors include the differences in depth and type of the bog layer, pH, time, temperature and microbiota. In addition, bacteria were isolated from the bog and the biosorption of I^-, SeO_3^{2-} and Cs^+ on six isolated bacterial strains was studied. The knowledge of the microbial effect on the I^-, SeO_3^{2-} and Cs^+ retention and migration in an acidic bog was one of the main objectives of this study. Specifically the aims were:

I. To investigate the different factors affecting the retention of I^-, SeO_3^{2-} and Cs^+ in the acidic boreal bog. These factors were the type of bog layer (moss, peat, gyttja and clay), time, pH and temperature. In addition, the applicability of kinetic models on the retention of I^- and SeO_3^{2-} was evaluated. (Manuscripts I, III, IV)
II. To evaluate the effect of microbiota on the retention of I^-, SeO_3^{2-} and Cs^+ in the bog. (Manuscripts I, III, IV)
III. To assess the ability of various bacteria isolated from the bog to retain I^-, SeO_3^{2-} and Cs^+. (Manuscripts I, II, III, IV)

7. Experimental

7.1. Sampling site, sampling and characterization of peat and bog water samples (Manuscripts I-IV)

Surface moss, subsurface peat, gyttja and clay as well as bog water samples for all publications I - IV were collected from an ombrotrophic, nutrient-poor bog, Lastensuo, located on western coast of Finland (61°17′ N, 21° 50′ S). Peat, gyttja and clay samples were taken from seven 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. In addition surface moss (mainly *Sphagnum* spp.) was collected. The upper layers from 0.5 m to 5.0 m consisted of peat with variable degrees of humification and the deeper layers from 5.5 – 6.0 m and 6.5 – 7.0 m of gyttja and clay, respectively. From each depth a total of six full 50 ml sterile centrifuge tubes of sampling material were aseptically taken using a peat corer with a nest length of 50 cm and a diameter of 15 cm. The samples were sealed in plastic and taken to the laboratory in cool bags. These samples were stored frozen at – 18 °C and thawed immediately before use and used as such. In addition samples were taken into 1 litre plastic bags, using a stainless steel peat corer with a smaller diameter, 5 cm, taken to the laboratory in cool bags and dried at room temperature. Additional separate samples were taken for the studies in anoxic conditions; an entire peat corer nest sample from the corer with a diameter of 15 cm was taken from each sampling depth, tightly sealed in plastic at the sampling site, brought to the laboratory in cool bags and transferred into an oxygen-free cabinet having a N_2 atmosphere. Only the innermost parts of the drill core samples were used for the experiments in anoxic conditions, as these parts were considered to have remained oxygen free during sampling and transportation. Bog water samples from the surface and the depths of 0.5 – 1.0 m, 2.5 – 3.0 m and 5.5 – 6.0 m were separated from the peat by filtering and a bog water model solution (Table 1) used in the determination of experimental batch K_d values was prepared based on the major cation and anion concentrations determined from the bog water samples using ICP-MS and IC (Table 1). Humification degree of the samples was determined using the von Post scale, loss on ignition (LOI, proxy for organic matter content) was determined by annealing the samples at 550°C and pH of the samples was determined in 0.01 M $CaCl_2$ solution (Table 2). The overall mineral composition and minerals in the < 2 µm fraction of the gyttja and clay layers were determined using XRD (Table 2) (Figure 2).

Table 1.Concentrations of major cations and anions in bog water and in the simulated bog water solution. For the measured concentrations the average concentrations from three drill core samples after filtration (0.45 µm) from surface, subsurface (0.5 – 1.0 m), middle peat (2.5 – 3.0 m) and gyttja (5.5 – 6.0 m) layers are shown and the range of the concentrations for each individual element are shown. In addition pH and DOC of the bog water from different layers are shown.

	Simulated bog water		**Measured concentrations in the bog water**			
			Average		Range	STDEV
	mg/L	meq/L	mg/L	meq/L	mg/L	mg/L
Na	3.91	0.17	3.91	0.17	3.08 – 5.10	1.49
Mg	0.47	0.04	0.47	0.04	0.12 – 0.76	0.48
K	2.03	0.05	2.03	0.05	1.18 – 4.74	1.13
Ca	1.98	0.10	1.98	0.10	1.24 – 2.70	1.36
Cl	5.34	0.15	4.53	0.13	3.39 – 5.66	0.82
NO3	2.40	0.04	3.61	0.06	3.12 – 4.12	0.78
SO4	8.17	0.17	4.03	0.08	2.94 – 6.46	1.63

	Bog water pH			**DOC in bog water**		
		STDEV	Range	mg/L	STDEV	Range
Surface	3.8	-	-	32	-	-
0.5 – 1.0 m	4.7	0.2	4.6 - 4.9	47	6	42 - 53
2.5 - 3.0 m	4.9	0.1	4.8 - 5.1	48	15	38 - 65
5.5 - 6.0 m	5.2	0.5	4.9 - 5.8	63	6	59 - 70

Table 2. The humification degree (von Post scale), loss on ignition (LOI, %) and pH of the surface moss, peat, gyttja and clay samples of Lastensuo bog. Minerals in the < 2 µm and > 2 µm fractions of gyttja and clay samples.

	Humification degree (H1 – H10)	**LOI (%)**	**pH**
Surface	H1	99.2	3.1
0.5 – 1.0 m	H3	99.5	3.1
2.5 – 3.0 m	H4	99.8	3.2
5.5 – 6.0 m	H6	95.0	4.0
6.5 – 7.0 m	Clay	15.3	5.3
	Minerals in < 2 µm fraction	**Minerals in > 2 µm fraction**	
5.5 – 7.0 m	Illite, clinochlore and kaolinite	Quartz, microcline, plagioclase, pyrite, Fe-hornblende	

7.2. Determination of model experimental K_d values of I^-, SeO_3^{2-} and Cs^+ and in situ K_d values of Cs^+ (Manuscripts I, III, IV)

The experimental distribution coefficients (K_d) for either dried, fresh or sterilized surface moss, subsurface peat, gyttja and clay samples were determined using batch experiments. For these experiments 0.5 g of each sample were weighted in sterile 50 ml centrifuge tube and 25 ml of simulated bog water was added. All samples were prepared in duplicate and sterile simulated bog water (Table 1) and aseptic work methods were used. The sample was incubated with simulated bog water for one week, during which time the ion concentrations were stabilized. The stabilization was ensured by measuring the cations using ICP-MS. After the stabilization 200 Bq/sample of $Na^{125}I$ (carrier free), 200 Bq/sample of $Na_2{}^{75}SeO_3$ (2.7×10^{-8} M SeO_3^{2-} carrier) or 1000 Bq/sample of $^{134}CsCl$ (0.2×10^{-6} M Cs carrier) were added to the solution. The samples were further incubated for 1 to 168 days, depending on the set of experiments, under constant stirring. After the selected incubation period, the samples were centrifuged at 20 000 rpm for 20 minutes (Beckman Coulter J-26 XPI, rotor JA-25.50), filtered through a 0.2 µm syringe filter (Supor membrane filter, Pall Corp., Port Washington, NY, USA), the solution pH was recorded and the solution was used for the gamma spectrometric determination of ^{125}I, ^{75}Se or ^{134}Cs activity. The experimental batch K_d values were calculated from the measuring results using Equation 15:

$$K_d = \left(\frac{(A_i - A_f)}{A_f}\right) \times \left(\frac{V(L)}{m(kg)}\right) \quad (15)$$

where A_i is the initial $^{125}I^-$, $^{75}SeO_3^{2-}$ or $^{134}Cs^+$ activity concentration of the sample (Bq/L), A_f is the final activity concentration of the solution (Bq/L), V is the solution volume (L) and m is the sample mass (kg).

To study the impact of microbiota on the sorption behaviour of I^-, SeO_3^{2-} and Cs^+, aliquots of fresh samples were sterilized by autoclaving the samples three times at 125 °C for 20 minutes. The second and third autoclaving was done 7 and 10 days after the initial autoclaving. These samples were used to determine experimental K_d values for bog material devoid of viable microorganisms and compared to the K_d values obtained for the unsterilized samples, representing the intact microbial population. For the sterilized samples the experimental K_d values were determined as described above using aseptic work methods.

As the change in pH affects the protonation of the functional groups found in the bog material, as well as the functionality of enzymes and microbiota possible participating the sorption behaviour of studied ions, the effect of pH on the sorption of I^-, SeO_3^{2-} and Cs^+ was studied. This was done by adjusting the pH of fresh, unsterilized batch sorption samples between pH 2 and pH 12 by addition of 0.1 M or 1 M HCl or NaOH, depending of desired pH. In these experiments an incubation period of 7 days was used, after which the samples were centrifuged and filtered as described above. The final pH of the samples was recorded and the samples were measured and the K_d values were calculated as described above.

Temperature typically affects the kinetics of enzymatic and other organic reactions and therefore two incubation temperatures, + 20 °C and + 4 °C, were used to examine the effect of temperature on the maximum sorption and sorption kinetics of I^- and SeO_3^{2-} (manuscripts I and III). These temperatures were expected to be found in a bog in the temperate climate prevailing in Finland. The temperature of +4 °C was selected to represent that found in the lower layers and + 20 °C the temperature of the upper layers during the summer months. Fresh, unsterilized as well as fresh sterilized samples of surface moss, subsurface peat, gyttja and clay and incubation periods between 7 to 84 days were used in these experiments. The PSO and Elovich kinetic models described in section 7.3 were applied for the data obtained from both temperatures.

The *in situ* distribution coefficients (K_d) for Cs^+ were calculated as the ratio of indigenous stable cesium concentration in the exchangeable form on the moss, peat and gyttja surfaces to the corresponding concentrations in bog water (manuscript IV). The concentration of exchangeable Cs was determined using 1M ammonium acetate (1M CH_3COONH_4 in 25% CH_3COOH, pH 4.5) extraction for the dried samples. One gram of moss, peat or gyttja and 30 mL of extraction solution was used and the samples were incubated at room temperature for 16 hours with constant stirring. After incubation, the extract was separated by centrifugation at 20 000 rpm for 20 minutes. The extract solution was filtered through a 0.2-µm Supor membrane filter (Pall Corp., Port Washington, NY, USA), the samples were diluted to 1:33 and Cs concentrations were determined using ICP-MS (Agilent 7500ce, Agilent Technologies, Inc., Santa Clara, CA, USA). The Cs concentrations of bog water samples filtered through a 0.2 µm Supor membrane filter (Pall Corp., Port Washington, NY, USA) were also determined using ICP-MS. Blank samples, consisting of extraction solutions without peat sample addition, were used in the extractions to detect possible sample contamination. All ICP-MS determinations were performed using two parallel samples and control samples with known concentrations were measured during all ICP-MS measurements.

Possible interfering cesium sorption on the 0.2-µm membrane filters was checked using a radioactive ^{134}Cs tracer. The ^{134}Cs tracer was filtered through two parallel 0.2-µm Supor membrane filters, the filters were washed with deionized water and the ^{134}Cs activity of the filters was measured using a semiconductor detector. The ^{134}Cs activity detected in the filters was within 0.02–0.03% of the original activity passed through the filters. Thus, the association of Cs with the membrane filters was considered negligible.

For the isotherm approach 10^{-9} M to 10^{-3} M $^{133}CsCl$ and 1000 Bq of ^{134}Cs per 0.5 g fresh peat sample in 25 mL of model bog water solution was used.

7.3. Modelling of Cs^+ sorption in bog samples and I^- and SeO_3^{2-} sorption kinetics in bog samples (Manuscripts I, III, IV)

The sorption kinetics can be described and predicted using different models (Chien and Clayton 1980, Sparks and Jardine 1983, Ho and McKay 2002, Yakout and Elsherif 2010, Aikpokpodion et al. 2013). In present study Pseudo-second-order (PSO) and Elovich models were used for the kinetic studies of I^- and SeO_3^{2-} sorption (Table 3). If a plot of q_t against $ln(t)$ gives a linear relationship the sorption is

considered to follow the Elovich model with a slope of $1/\beta$ and an intercept of $1/\beta ln(\alpha\beta)$. Correspondingly for the PSO model, if the sorption follows the PSO model, a plot of t/q_t against t gives a linear relationship with a slope of $1/q_e$ and an intercept of $1/kq_e^2$.

The Freundlich sorption isotherm (Campbell and Davies 1995, Kamei-Ishikawa et al. 2008) was tested to fit the experimental batch K_d data of Cs^+ obtained for the fresh surface moss, peat, gyttja and clay samples (Table 3). If the plot *log (x/m)* versus *log C* yields a straight line, the sorption process conforms to the Freundlich model.

Table 3. The kinetic and sorption models used to describe the sorption kinetics of I^- and SeO_3^{2-} and the sorption of Cs^+ in surface moss, peat, gyttja and clay.

Model	The equation		Boundary conditions
Elovich	$q_t = \frac{1}{\beta}\ln(\alpha\beta) + \frac{1}{\beta} lnt$	q_t = amount of adsorbate adsorbed at time t (L/kg DW) α = initial sorption rate (L/kg DW d) β = desorption constant (kg DW/L) t = time (d)	$q_t = 0$ at t = 0 $q_t = q_t$ at $t = t$
Pseudo-second-order	$\frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{t}{q_e}$	q_t = amount of adsorbate adsorbed at time t (L/kg DW) q_e= sorption capacity at equilibrium (L/kg DW) $h = kq_e^2$ = initial sorption rate (L/kg DW d) t = time (d)	$q_t = 0$ at t = 0 $q_t = q_t$ at $t = t$
Freundlich	$\log(x/m) = logK_f + {}^1/_N\, logC$	C = the concentration of adsorbate in solution (mg L^{-1}) (x/m) = concentration in sample (mg kg^{-1} DW^{-1}) K_f = constant related to adsorption capacity ($g^{1-1/N}kg^{-1}L^{1/N}$) N = constant related to adsorption intensity.	

7.4. Isolation, characterization and identification of bacteria from Lastensuo bog (Manuscript II)

Bacteria used in manuscripts II, III and IV were isolated from the surface moss, peat, gyttja and clay samples of the Lastensuo bog. For the isolation one gram of fresh sample was suspended in 10 ml of sterile water, homogenized by vortexing and serially diluted using sterile water in 10-fold steps to dilution up to 10^{-5}. From dilutions 10^{-3} and 10^{-5} aliquots of 100 µl were spread on Plate Count Agar (PCA, 0.5 % Peptone + 0.25 % Yeast extract + 0.1 % Glucose + 1.5 % agar, Merckoplate®) and the plates were incubated at 20 °C for two weeks in the dark (Burlage et al. 1998, Pepper et al. 2009). After the two weeks incubation time pure cultures were prepared from each colony on PCA. The cells from the pure cultures were gram stained and examined using light microscope with 1000-fold magnification (Nikon ECLIPSE E200) (Pommerville 2011). Six bacterial strains from different peat layer depths were selected for further characterization; three gram negative and three gram positive strains. The catalase and oxidase activity of the colonies was tested using 3 % hydrogen peroxide and 1 % Kovács oxidase reagent. The gram negative, oxidase negative bacteria were further characterized using RapID™ ONE system (Remell), RapID™ NF Plus systems (Remell) and ERIC® electronic code.

The bacterial isolates were identified using 16S rRNA gene sequencing. The bacterial DNA was extracted using the NucleoSpin Soil DNA extraction kit (Macherey-Nagel). Primers fD1 and rD1 were used for the PCR amplification and the PCR was performed using Mastercycler Gradient (Eppendorf, Germany). Agarose gel electrophoresis was used to verify approximately 1.5 kb PCR products. The PCR products were sent for purification and sequencing to Macrogen Inc., Korea. The sequencing was conducted in both directions using primers fD1 and rD1.

Geneious Pro v.6.1.6. (Biomatters Ltd., Auckland, New Zealand) was used for the phylogenetic analyses of the sequences. The Blastn tool in Geneious Pro was used to compare the obtained 16S rRNA sequences to the sequences deposited in Genebank in order to identify the isolated bacteria (Altschul et al. 1997). MUSCLE alignment tool in Geneuois Pro was used to align the 16S rRNA gene sequences of the isolated bacteria with the closest matching sequences from Genebank as well as relevant reference sequences (Edgar 2004). A phylogenetic tree was calculate from the alignments using the PhyML tool (Guindon and Gascuel 2003) and the JC96 substitution model (Jukes and Cantor 1969) and the topology of the tree was tested by bootstrap analyses of 1000 random resamplings. The sequences have been deposited in Genebank under accession numbers KP100420 – KP100425.

7.5. Bacterial culture conditions and performance of uptake experiments (Manuscripts II, III, IV)

Bacteria isolated from peat were aerobically cultured on sterile PCA plates at 20 °C in the dark and colonies were transferred onto new plates weekly. The uptake of I^-, SeO_3^{2-} and Cs^+ by the isolated bacteria was studied using a batch method. The uptake experiments were carried out using $Na^{125}I$, $Na_2{}^{75}SeO_3$ or $^{134}CsCl$ in one (medium A, manuscript IV), two (media A and B, manuscript III) or four (media A, B, C and D, manuscript II) different liquid media. Medium A consisted of 1 % Tryptone and 0.5. % NaCl, medium B of 1 % Yeast extract and 0.5 % NaCl, medium C of 0.5 % Peptone and 0.25 % Yeast extract with 0.1 % Glucose. Medium D was the same as medium C, except without the addition of glucose. Medium C corresponded to the medium used in the initial isolation of the bacteria and medium D was chosen to study the effect of glucose on the bacterial uptake of iodide (manuscript II). For the uptake experiments, bacterial suspensions were prepared by adding isolated bacterial colonies from the growth plates into weighted sterile water until the turbidity of the solution corresponded to a McFarland standard nro 6. The suspensions were weighted and 2 ml bacterial suspension was added to 5 ml of broth solution A, B, C or D, depending on the set of experiments and $Na^{125}I$, $Na_2{}^{75}SeO_3$ or $^{134}CsCl$ was added to the solution. For $Na^{125}I$ and $Na_2{}^{75}SeO_3$ activity of 200 Bq/suspension was used and for $^{134}CsCl$ 1000 Bq was used per suspension. The solutions were incubated for different incubation periods 1 day (manuscripts III and IV), 7 days (manuscripts II – IV)) or 14 days (manuscripts III and IV), depending on the set of experiments, in the dark. Three different incubation temperatures, +4 °C, + 20 °C and + 37 °C were used. After incubation the suspensions were filtered through a 0.2 µm sterile membrane filter and ^{125}I, ^{75}Se or ^{134}Cs activity was measured from the resulting solution using a NaI(Tl) detector. To assure that no sorption of I^-, SeO_3^{2-} and Cs^+ on laboratory equipment, filters or nutrient broth solutions occurred,

suspensions without added bacteria were prepared and measured accordingly and initial activity added was obtained from these solutions. The removal of I^-, SeO_3^{2-} and Cs^+ from the solution by bacterial cells was calculated from the difference between initial and final I^-, SeO_3^{2-} or Cs^+ concentration in the nutrient solution and expressed as distribution coefficient (K_d) calculated in similar manner as described above in section 7.2. For the calculations the dry weight of bacteria was determined in 105 °C. The samples were incubated for one week at 20 °C in the dark, after which the samples were filtered and measured as described above.

Isolated bacteria were in addition used to study the bacterial effect on the removal of SeO_3^{2-} and Cs^+ (manuscripts III and IV) from the solution when sterilized moss, peat, gyttja or clay and bacteria were present. 2 ml of similar bacterial suspension as prepared for the uptake experiments from broths A, B, C and D and 0.5 g of sterilized surface moss, peat, gyttja or clay were added to 25 ml of sterilized simulated bog water with 200 Bq of $Na_2{}^{75}SeO_3$ or 1000 Bq of $^{134}CsCl$. The samples were incubated at 20°C in the dark for seven days, centrifuged, filtered and measured thereafter as described in section 7.2. The removal of selenite or cesium from the solution was expressed as a percentage of the total added concentration and compared to the removal of SeO_3^{2-} or Cs^+ when only sterilized surface moss, peat, gyttja or clay were used.

7.6. Preparation of microbial extract, bacterial cell enumeration and microbial enzyme determinations (Manuscript I)

To study the effect of microbes on the uptake of I^- in the bog samples, a microbial extract was prepared from the fresh surface moss sample by adding fresh surface peat to sterile MilliQ water in a mass-to-volume proportion of 1:1. The sample was incubated at +20 °C for five days in the dark. After incubation the peat was let to settle and the supernatant containing the microbes was removed by pipetting. Thereafter, 1 mL of the supernatant i.e. the obtained microbial extract and 24 mL of sterile model bog water solution were used for the recolonization experiments of sterilized samples. Otherwise, the samples were prepared as described above in section 7.2.

Bacterial counts were determined from the batch sorption experiments of I^-. For the enumeration of bacterial cells present in the batch sorption experiment samples, PCA (Merckoplate®) was used. After the incubation and centrifugation of the batch sorption experiment samples, a one-third aliquot of the remaining solid sample was used to enumerate the viable colony-forming bacteria present in the samples. Ten millilitres of sterile water was added to the solid sample and the sample was homogenized by vortexing. The resulting suspension was serially diluted in sterile water in 10-fold steps using 100 µl of homogenized solution per PCA plate. The aliquots from dilutions of 10^{-3} and 10^{-5} were spread on PCA and the plates were incubated at 20 °C for one week in the dark, which after the colonies were counted.

Microbial peroxidase enzyme activities were determined from the batch sorption experiment samples of I^-. After the incubation and centrifugation of the batch sorption samples (section 7.2.), a one-third aliquot of the remaining solid was used for the peroxidase determinations. For these determinations, 15 ml of sodium acetate buffer (pH 5) was added and the sample was mixed. A 2-ml aliquot was

taken from the slurry and 2 ml of 5 mM L-DOPA substrate and 0.2 ml of 0.3 % H_2O_2 was added. The samples were incubated at room temperature for 60 min, which after the samples were centrifuged at 6530 xg for 15 min at 4 °C. The absorbance of the supernatant was recorded at 450 nm using a spectrophotometer. In addition phenol oxidase determinations were conducted similarly to peroxidase enzyme determinations with the exception of no H_2O_2 addition. Phenol oxidase activity was needed to calculate the peroxidase activities, as the absorbance of phenol oxidase activity is subtracted from the absorbance of peroxidase activity. A molar extinction coefficient of 1.66 μmol^{-1} was used in the calculations.

8. Results

8.1. The effect of sampling depth and time on the sorption of I^-, SeO_3^{2-} and Cs^+ and the application of sorption kinetics models (Manuscripts I, III and IV)

The sampling depth affected the experimental batch K_d values of the anions I^- and SeO_3^{2-} and the values decreased as a function of the sampling depth (Figure 12A and B). For cationic Cs^+ the experimental batch K_d values were relatively constant in the surface moss, subsurface peat, middle peat and gyttja layers at incubation times between 7 – 84 days (geometric mean 60 L/kg DW). In the clay layer (6.5 – 7.0 m) a notably 30-fold increase in the experimental K_d values of Cs^+ were observed (geometric mean 3200 L/kg DW at 7 – 84 days) (Figure 12C).

For I^- clearly the highest K_d values were measured in the surface moss. In this layer the geometric mean K_d value for I^- was 4800 L/kg and decreased to 1500 L/kg DW in the partly decomposed peat at 0.5–1.0 m. A further decrease in the I^- sorption was observed in the gyttja and particularly in the clay layer, in which the sorption of I^- decreased 50-fold compared to that of the surface moss (Figure 12A). Similarly also for SeO_3^{2-} the highest K_d value was observed in the fresh surface moss sample (geometric mean 6600 L/kg between 7 – 84 days). In the 0.5–3.0 m layer the corresponding value was 4500 L/kg DW (geometric mean) and in the gyttja (5.5–6.0 m) and clay (6.5–7.0m) layers 2100 L/kg DW.

Incubation time affected the sorption of I^- and SeO_3^{2-} in all studied layers. For I^- the maximum sorption in fresh samples was observed after 56 days of incubation (13 000 L/kg DW) and for SeO_3^{2-} the sorption was observed to increase until 56 days of incubation in all layers, which after the K_d values remained relatively constant. For SeO_3^{2-} the highest mean K_d values, 7500 L/kg DW and 8800 L/kg DW after 56 – 84 days of incubation, were observed in the fresh surface moss and 2.5 – 3.0 m peat samples, respectively (Figure 12B). In the depths from 0.5 – 1.0 m and 5.5 - 6.0 m the corresponding mean K_d value after 56 – 84 days of incubation was 7400 L/kg DW and in the clay layer 2700 L/kg DW. Only a relatively minor increase from 45 L/kg DW (geometric mean) after 1 day incubation to 85 L/kg DW after 84 days of incubation was observed in the K_d values of Cs^+ in the peat and gyttja layers (Figure 12C). As incubation was continued to 168 days, the sorption of Cs^+ in these layers was recorded to decrease to 55 L/kg DW (geometric mean). In the clay no effect of time on the sorption was observed and the Cs^+ K_d values remained stable after seven days of incubation (Figure 12C).

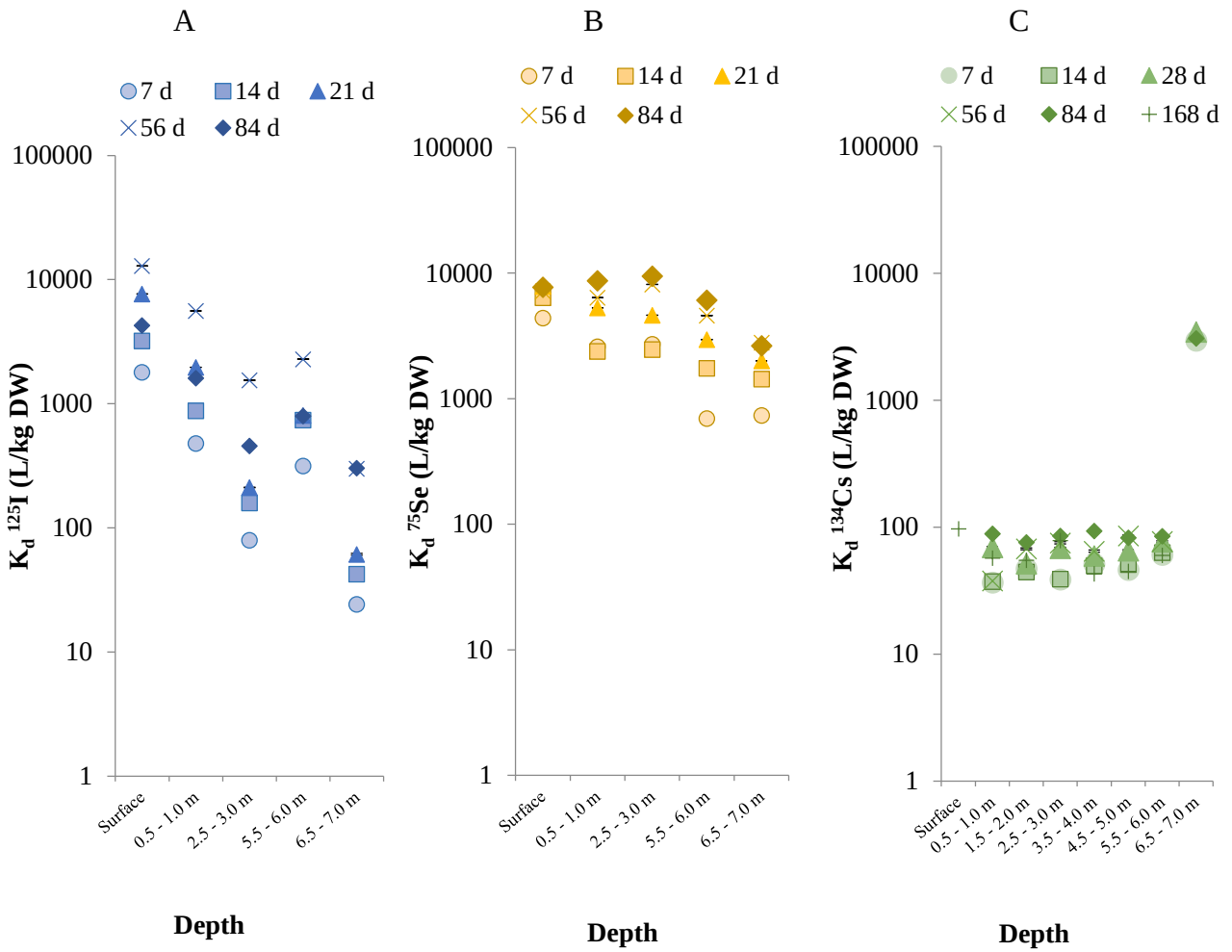


Figure 12. Sorption of I^- (A), SeO_3^{2-} (B) and Cs^+(C) as a function of sampling depth in the surface moss, peat, gyttja and clay of Lastensuo bog at + 20 °C. In (A) and (B) incubation times 7 – 84 days and in (C) 7 – 168 days are shown. All values represent geometric means of two or three parallel determination. The uncertainty (geometric standard deviation) is smaller than the symbol. In (A) and (B) fresh, unsterilized samples were used and in (C) dried, unsterilized samples.

The Elovich and PSO kinetic models were fitted to the experimental batch K_d data (Shetaya et al. 2012) to model the sorption kinetics of I^- and SeO_3^-. K_d versus *lnt* (Elovich model with K_d) (Shetaya 2011) and t/K_d versus t (PSO model with K_d) (Shetaya 2011), with time in days, were plotted for I^- and SeO_3^- and the r^2 values for the linear equations were determined (Table 4). The Elovich model plots gave a better overall fit for both I^- and SeO_3^{2-}, compared to the PSO model and the initial sorption rate α (L/kg DW d) and the desorption constant β (kg DW/L) were calculated from the intercept and slope of the Elovich plots (Table 4).

Temperature affected the sorption kinetics of I^- and SeO_3^{2-} and the obtained values of α and β varied as the incubation temperature was changed (Table 4). When the incubation temperature was increased from +4 °C to +20 °C, the average β value of I^- decreased from 13×10^{-3} to 2.1×10^{-3} kg DW/L and the average α value increased from 78 L /kg DW d to 300 L/kg DW d. Similarly, an increase in the solution temperature from +4 °C to +20 °C increased the average α value of $SeO_3^{2-}$from 330 L/kg

DW d to 4200 L/kg DW d and simultaneously the average β value decreased from 4.2×10^{-3} to 0.7×10^{-3} kg DW/L. This means that an increase in temperature from +4 °C to +20 °C, increases the sorption rate (L/kg DW d) of both I^- and SeO_3^{2-}.

Table 4. Calculated α, β and r^2 values from the Elovich equation with K_d for the sorption of I^- and SeO_3^{2-} (T = + 4°C and +20 °C). α is the initial sorption rate (L/kg DW d) and β is the desorption constant (10^{-3} kg DW/L).

	I^-						**SeO_3^{2-}**					
	$\alpha_{20\,°C}$	$\alpha_{4°C}$	$\beta_{20\,°C}$	$\beta_{4\,°C}$	$r^2_{20°C}$	$r^2_{4°C}$	$\alpha_{20\,°C}$	$\alpha_{4°C}$	$\beta_{20\,°C}$	$\beta_{4\,°C}$	$r^2_{20°C}$	$r^2_{4°C}$
Surface	930	230	0.2	1.2	0.95	0.99	18600	380	0.9	0.4	0.63	0.97
0.5–1.0 m	340	51	0.4	4.7	0.90	0.85	780	200	0.4	7.7	0.88	0.74
2.5–3.0 m	77	42	1.4	35	0.80	0.87	740	150	0.3	7.8	0.93	0.91
5.5–6.0 m	140	44	1.1	3.0	0.91	0.87	399	45	0.5	3.2	0.98	0.63
6.5–7.0 m	16	22	7.4	20	0.82	0.99	340	850	1.2	2.0	0.93	0.62

8.2. The in situ K_d values and Freundlich isotherm of Cs^+ (Manuscript IV)

The *in situ* K_d values of Cs^+ were calculated from bog water Cs^+ concentrations and the concentrations of exchangeable Cs^+ obtained from the 1 M ammonium acetate extraction and were compared with the experimental batch K_d values obtained after 168 days of incubation (Figure 13). Clearly highest *in situ* K_d values (geometric mean 9040 L/kg DW) were observed in the surface moss layer. In the peat and gyttja layers (5.5–6.0 m), the *in situ* K_d values ranged from 420 L/kg DW (2.5–3.0 m) to 740 L/kg DW (0.5–1.0 m). In the gyttja layer, the recorded *in situ* K_d was 530 L/kg DW.

The *in situ* K_d values were about one order of magnitude higher than the batch K_d values and an even greater difference was observed in the surface moss layer. In the surface moss the batch K_d value was only 1.1% of the value recorded for the *in situ* approach. In the other studied layers, the batch K_d values were 7.7% (0.5 – 1.0 m), 19% (2.5 – 3.0 m) and 11% (5.5 – 6.0 m) of the *in situ* values.

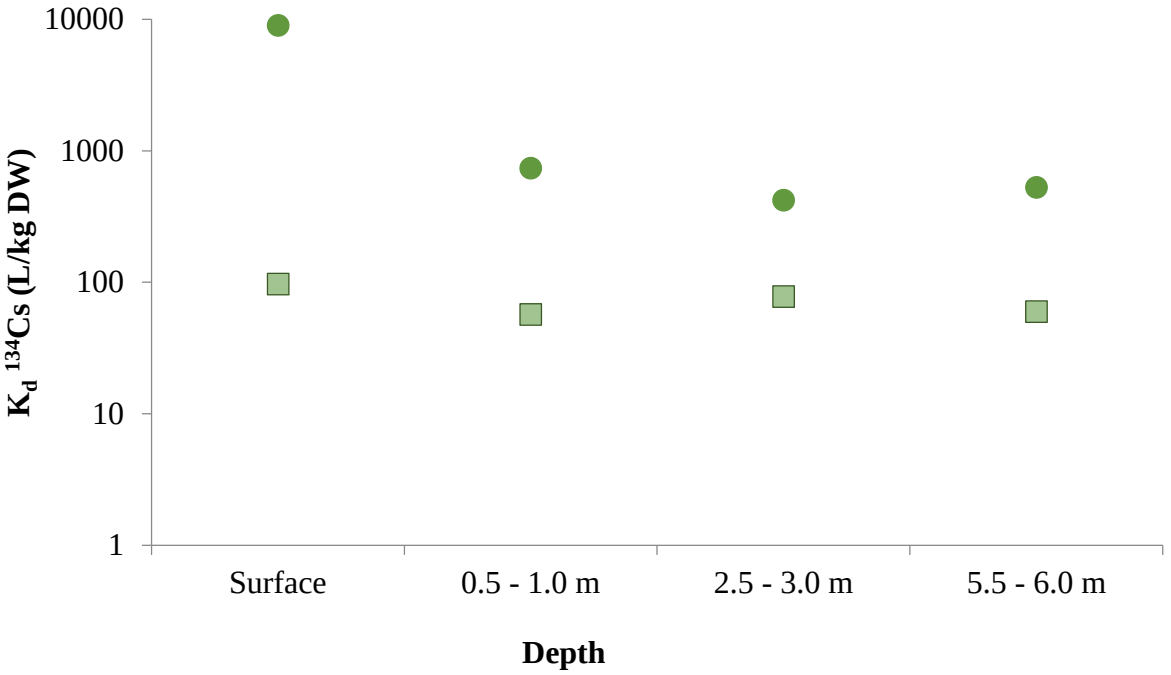


Figure 13. In situ K_d values of Cs^+ and experimental batch K_d values of Cs^+ after 168 days of incubation (dried samples). Circle = in situ values, square = batch values. The in situ values represent the geometric means of duplicate analyses from three different samples (n = 6), the batch values are geometric means of duplicate analyses and the uncertainty is smaller than the symbol (geometric standard deviation).

The isotherm approach was used to model the sorption of Cs^+ on fresh surface moss, peat, gyttja and clay samples (Figure 14). *Log (x/m)* versus *logC* (Freundlich model) was plotted and the r^2 values for the linear equations were calculated (Figure 15). The average r^2 value for all layers was 0.99 with a range from 0.98 to 1. The N values obtained from the Freundlich model were between 1.0 and 1.1 for the surface moss, peat and gyttja layers and 1.4 for the clay layer. The K_f values were between 2.5 – 9.5 $\times 10^{-5}$ $g^{1-1/N}kg^{-1}L^{1/N}$, with lowest values found for the clay layer and highest for the peat layer from 2.5 – 3.0 m. However as the N values differ from 1, it is not possible to compare the K_f values obtained for the different layers. The lower K_f value recorded for the clay layer compared to the upper layers is contradictory to the Cs K_d values recorded for the clay layer above and is due to the extrapolation to a higher equilibrium concentration of cesium (N>1 in the clay layer). At lower cesium concentrations ($C_{eq} < 4\times10^{-5}$ g/L) the deviation of N from 1 in the clay layer decreased (N = 1.1) and the K_f values increased to 4.5×10^{-4} $g^{1-1/N}kg^{-1}L^{1/N}$.

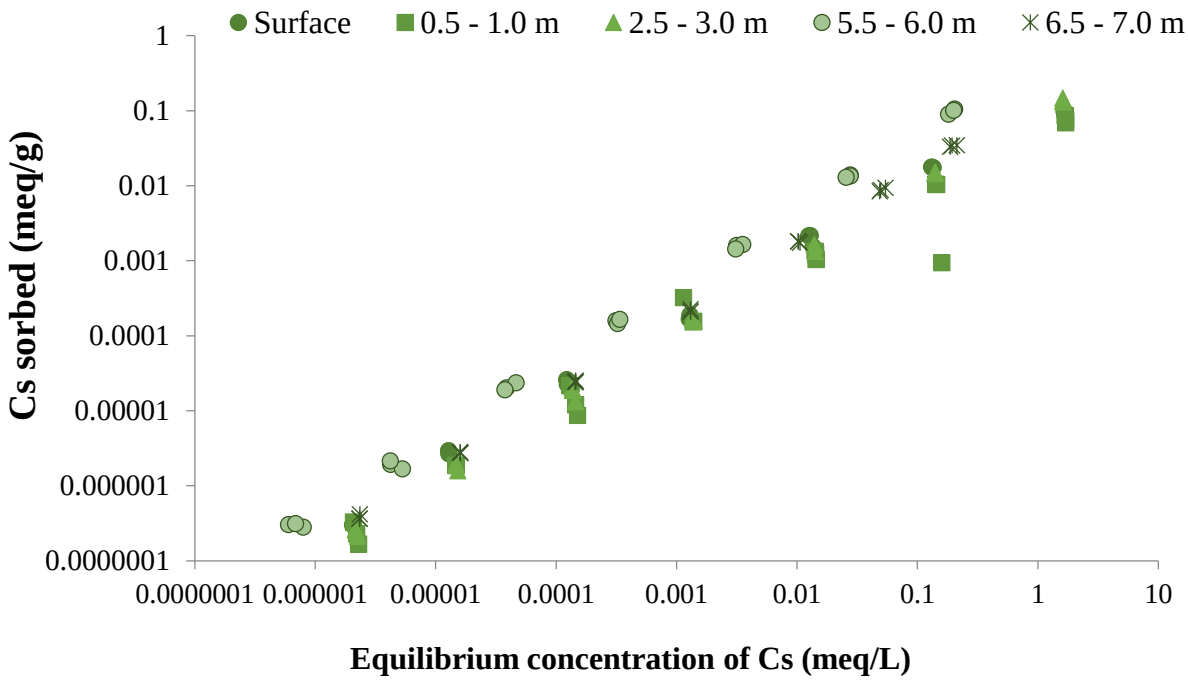


Figure 14. The amount of Cs^+ sorbed (meq/gDW) as a function of equilibrium concentration of Cs^+ in solution (meq/L) (fresh samples).

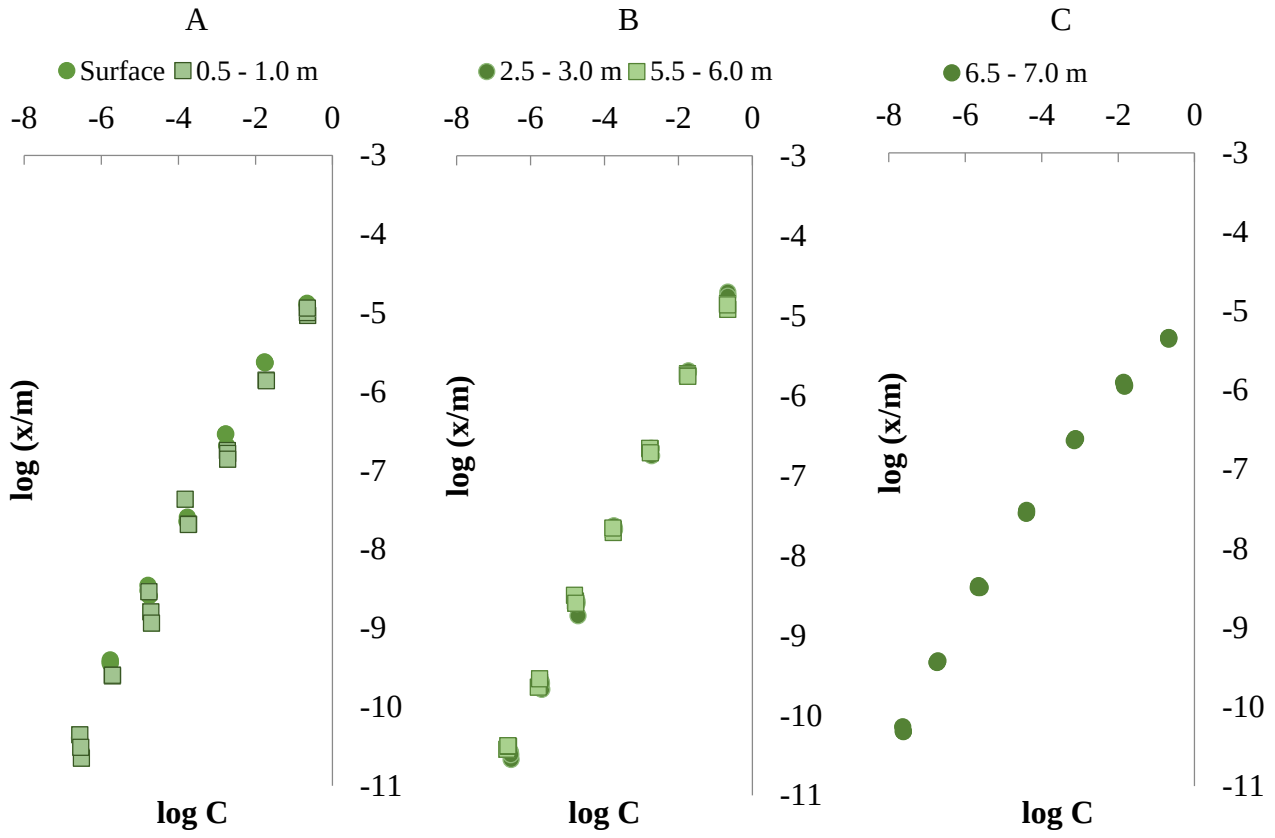


Figure 15. The Freundlich isotherms for Cs^+ sorption in surface moss and 0.5 – 1.0 m layer (A), in 2.5 – 3.0 m and 5.5 – 5.0 m layer (B) and in 6.5 – 7.0 m layer (C) (fresh samples).

8.3. The effect of pH on the sorption of I^-, SeO_3^{2-} and Cs^+ (Manuscripts I, III and IV)

The effect of pH on the sorption of I^-, SeO_3^{2-} and Cs^+ was studied using experiments with pH adjusted between pH 2 and pH 12 and an incubation period of 7 days. The pH was recorded at the end of the incubation period and K_d was presented as a function of the final pH (Figure 16). For the anions, I^- and SeO_3^{2-}, the maximum sorption in the surface moss, peat and gyttja (and for SeO_3^{2-} also in clay) was detected between pH ~3.0–5.0 (Figure 16A and Figure 16B). For both I^- and SeO_3^{2-} a clear increase in the batch K_d values was observed between pH 2 and pH 3 – 4 in the 0 – 6.0 m layers, where after the K_d values of I^- clearly decreased with increasing pH. For SeO_3^{2-} a plateau was detected between pH 3 and 6.5, which after the K_d values decreased to a mean of 10 L/kg DW at pH above 10. In the case of I^- the clay layer (6.5–7.0 m) behaved quite differently from the upper layers. The maximum K_d was observed at pH 2 and a sharp 7.5-fold decrease in the K_d values between pH 2 and 4 was recorded, where after a plateau was observed (Figure 16A). The mean K_d (geometric mean) of I^- found in the clay layer above pH 4 was 13 L/kg DW.

As a cation, Cs^+ behaved quite differently from anionic I^- and SeO_3^{2-}. For the upper peat (0.5–3.0 m) and gyttja (5.5–6.0 m) layers, the maximum sorption of Cs^+ was detected in the alkaline region between pH ~7.0–9.5 (Figure 16C) and in all these layers, a substantial increase in the batch K_d values was observed between pH 2 and pH 7. A plateau was recorded between pH 7 and pH 10. In the clay layer (6.5–7.0 m), Cs^+ behaved somewhat differently from the upper layers. The maximum K_d was observed at pH 8.9, but the increase between pH 2 and pH 7 was less steep compared to the increase observed for the upper layers (Figure 16C).

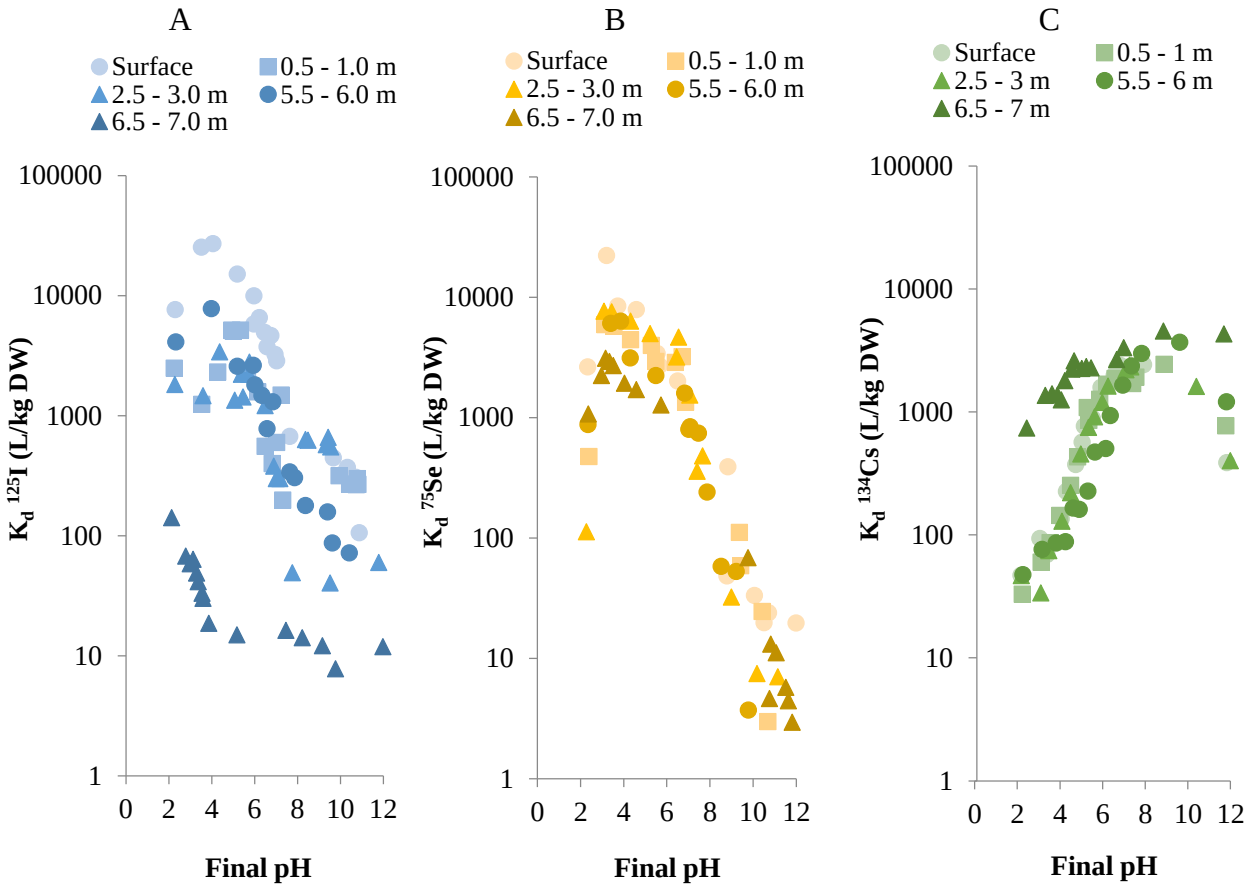


Figure 16. Sorption of I^- (A), SeO_3^{2-} (B) and Cs^+ (C) in fresh samples as a function of adjusted pH, measured at the end of the incubation period. Incubation period 7 days, T = 20 °C.

8.4. The effect of sterilization on the sorption of I^-, SeO_3^{2-} and Cs^+ and the effect of anoxic conditions on the sorption of I^- and SeO_3^{2-} (Manuscripts I, III and IV)

The effect of sterilization on I^- and SeO_3^{2-} sorption on the surface moss, peat, gyttja and clay samples was investigated at two temperatures, +20 °C and +4 °C. For Cs^+ a temperature of +20 °C was used. For both anions, I^- and SeO_3^{2-}, little or no sorption at all was observed after sterilization of moss, peat and gyttja samples (Figure 17A and B, Figure 18A and B). In the case of SeO_3^{2-} sorption was observed on these layers only after longer incubation periods of 56 or 84 days at +20 °C and at + 4 °C no sorption of SeO_3^{2-} was observed regardless of the incubation time in these layers (Figure 17B and Figure 18B). At + 20 °C even with longer incubation periods only on average 1.0 % of the SeO_3^{2-} sorption observed in the unsterilized samples was measured in the sterilized samples. For I^- the geometric means of the K_d values of sterilized surface moss, peat and gyttja samples were only on average 6% of the values recorded for the unsterilized samples and in the case of the surface moss samples, the effect of sterilization on the I^- sorption was particularly clear. A 500-fold and a 200-fold drop in the I^- sorption was observed after sterilization of the surface moss samples at +20 °C (Figure 17A) and at +4 °C (Figure 18A), respectively.

In the clay layer, sterilization affected the sorption of SeO_3^{2-} considerably less. At + 20 °C the K_d values of SeO_3^{2-} obtained for the sterilized clay were on average 18 % of the values recorded for the unsterilized samples after 7 – 84 days of incubation and 30 % at + 4 °C. For I^- a notable 200-fold decrease in K_d values of clay samples was observed after sterilization in the incubations at both +20 °C and +4 °C (Figure 17A, Figure 18B).

ANOVA analysis for logtransformed data confirmed the statistical difference in the I^- and SeO_3^{2-} sorption between unsterilized and sterilized samples at both temperatures in all layers ($F_{(20\ °C,\ I^-)} = 55.569$, $F_{Crit(20\ °C,\ I^-)} = 4.043$, $p_{(20\ °C,\ I^-)} < 0.001$, $F_{(4\ °C,\ I^-)} = 43.455$, $F_{Crit(4\ °C,\ I^-)} = 4.043$, $p_{(4\ °C,\ I^-)} < 0.001$, $F_{(20\ °C,\ SeO3^{2-})} = 170.0$, $F_{Crit(20\ °C,\ SeO3^{2-})} = 4.043$, $p_{(20\ °C,\ SeO3^{2-})} = 2.18\times10^{-17}$, $F_{(4\ °C,\ SeO3^{2-})} = 91.6$, $F_{Crit(4\ °C,\ SeO3^{2-})} = 4.043$, $p_{(4\ °C,\ SeO3^{2-})} = 1.05\times10^{-12}$).

For Cs^+ the experimental batch K_d values were found on average 38% lower in the sterilized samples compared to the values recorded for the unsterilized samples (Figure 17C), but the K_d values for sterilized and unsterilized samples did not differ statistically from each other ($F = 1.721$, $F_{Crit} = 4.196$).

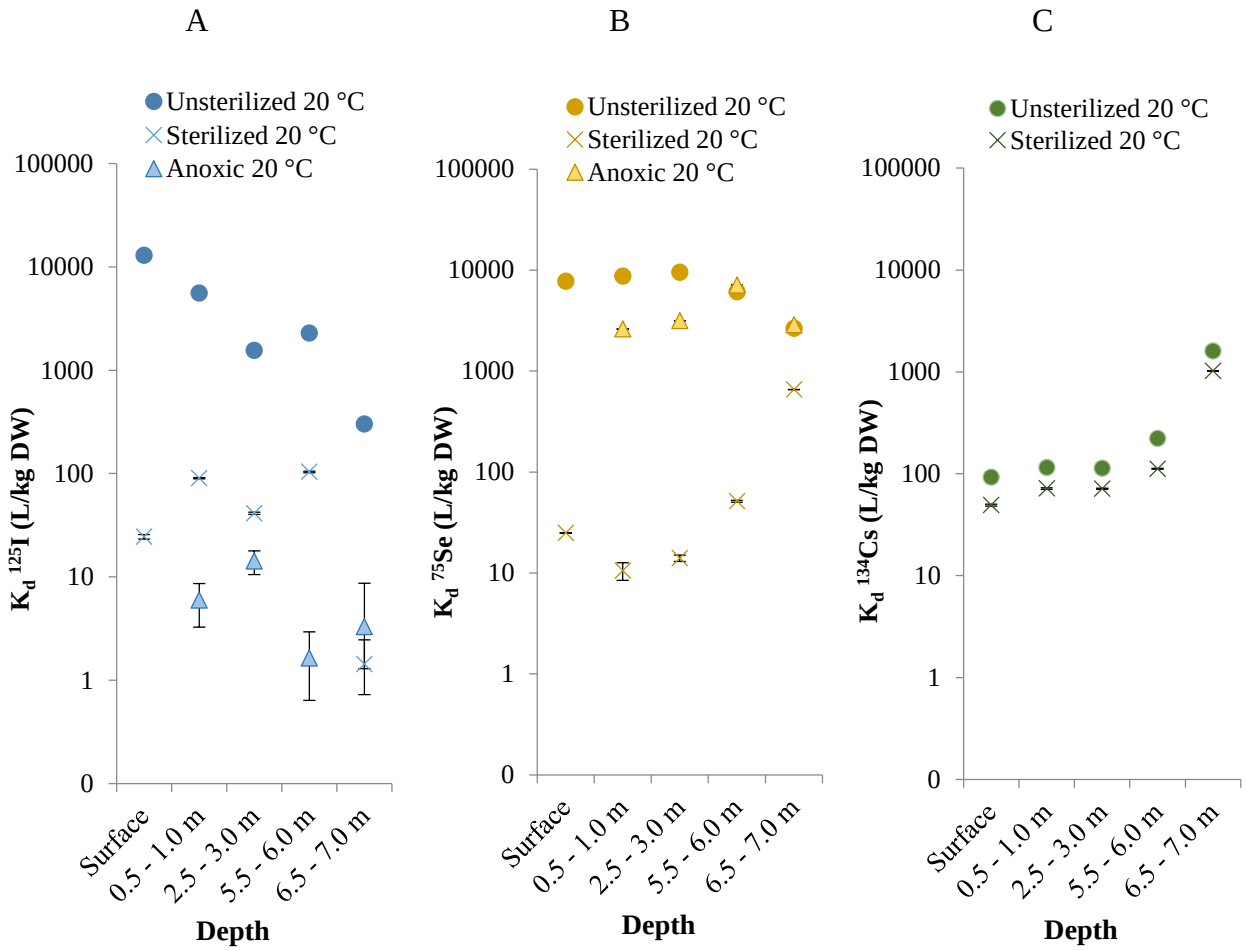


Figure 17. Experimental batch K_d values of I^- (A), SeO_3^{2-} (B) and Cs^+ (C) at +20 °C as a function of depth in unsterilized and sterilized fresh samples and in anoxic conditions (A and B). In (A) incubation time = 56 days, in (B) incubation time = 84 days, in (C) incubation time = 7 days. The values represent the geometric means of duplicate (A and B) or triplicate (C) analyses and the uncertainty bars indicate the geometric standard deviation. The absence of bars indicates that the uncertainty is smaller than the symbol.

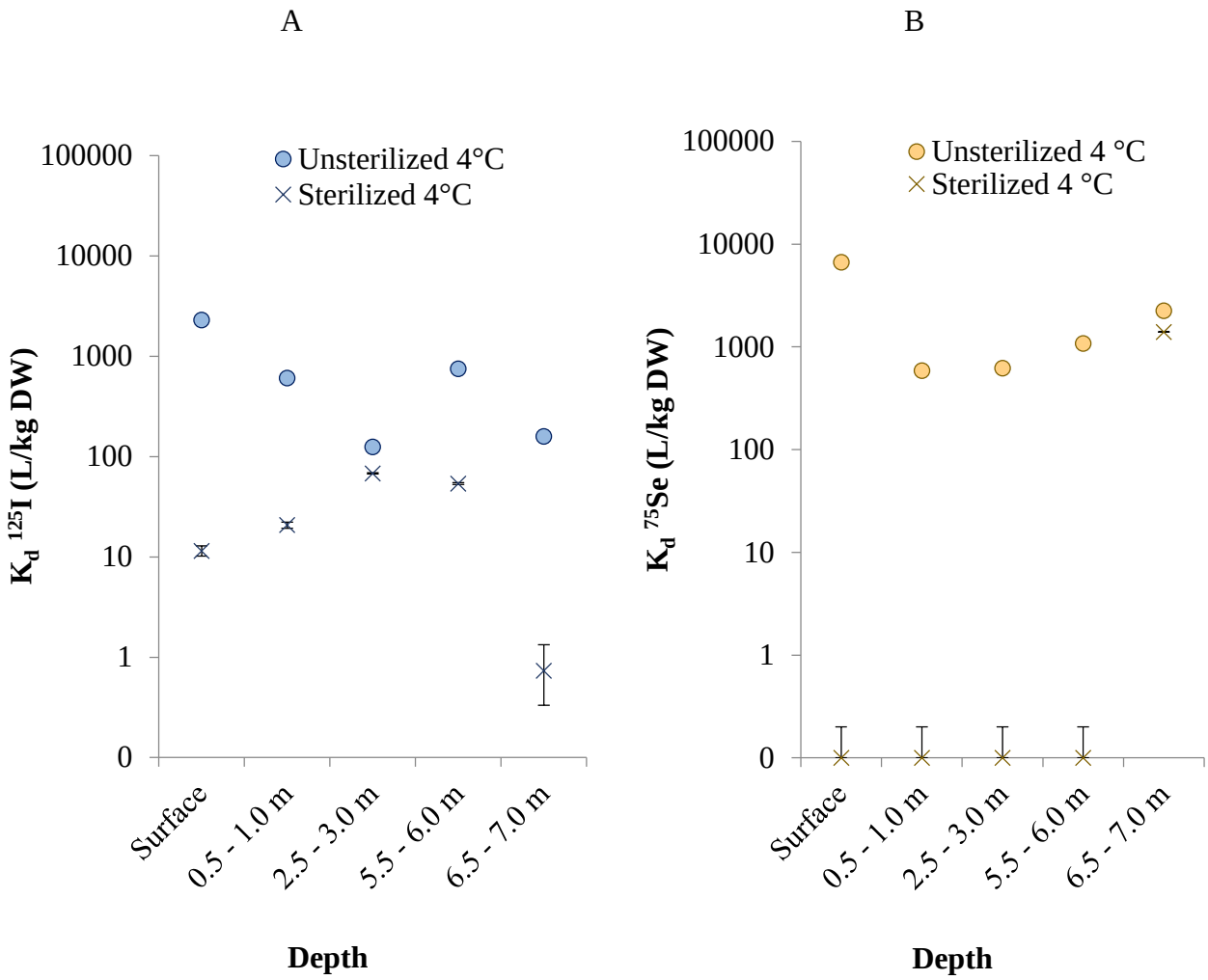


Figure 18. Experimental batch K_d values of I^- (A) and SeO_3^{2-} (B) at +4 °C as a function of depth in unsterilized and sterilized fresh samples. Incubation time 56 days in (A) and 84 days in (B). The uncertainty bars indicate the geometric standard deviation. The absence of bars indicates that the uncertainty is smaller than the symbol.

In the anoxic conditions the sorption of I^- was generally low in all layers, on average only 2% of the sorption observed under oxic conditions (Figure 17A). For SeO_3^{2-} the effect of anoxic conditions was clearly smaller (Figure 17B). After 7 – 84 days of incubation in the anoxic conditions the sorption of SeO_3^{2-} in the 0.5 – 3.0 m layers was on average 12 % of the sorption found in the oxic conditions. In contrast to the upper layers as well as to the sorption of I^-, the sorption of SeO_3^{2-} in the gyttja layer (5.5 – 6.0 m) was 20 % higher in the anoxic conditions compared to the sorption under oxic conditions after 84 days of incubation. In the clay layer (6.5 – 7.0 m) the sorption of SeO_3^{2-} in anoxic conditions was 50 % from the sorption observed in oxic conditions, over incubation times between 7 – 21 days, but over 56 – 84 days of incubation the sorption of SeO_3^{2-} remained virtually constant (geometric mean 2800 L/kg DW) both under anoxic and oxic conditions.

8.5. Correlation between microbial peroxidase activity and bacterial counts and the sorption of I^- and the effect of recolonization of sterilized samples with microbes on the sorption of I^- (Manuscript I)

The correlation between microbial peroxidase activities in the sorption experiment samples and I^- K_d value was examined. This was done to study the possible enzymatically catalysed oxidation of I^- into molecular iodine (I_2) or hypoiodous acid (HIO) prior to its incorporation into the organic matter. In addition, the amount of bacterial cells was determined from the sorption experiment samples to examine the correlation between the number of bacteria present in the sample and the I^- K_d value.

The I^- K_d values were found to correlate positively with peroxidase activity in the moss, peat and gyttja layers at +20 °C (R 0.68, $p < 0.01$), but not in the clay layer or at +4 °C. The viable bacterial cell counts in the sorption experiment samples correlated positively with the I^- K_d values at +4 °C, (R 0.50, $p < 0.05$) for all incubation times. The correlation was strongest after one week of incubation (R 0.93, $p < 0.05$), and decreased thereafter as the incubation time was increased to two weeks. After two months of incubation, no positive correlation was seen. The correlation between viable cell counts and I^- batch K_d values at +20 °C could not be determined, because in this temperature the colony forming units on all culture plates were too numerous to count.

The sterilized surface moss, peat and gyttja samples were recolonized with microbes by incubating the samples with corresponding fresh samples (Figure 19A) or microbial extract (Figure 19B) and $Na^{125}I$. The addition of fresh sample onto the sterilized sample increased the sorption of iodide in the surface moss and gyttja layers on average by a factor of 10, 20 and 35, respectively, after the addition of 25 m-%, 50 m-% and 75 m-% of fresh peat (Figure 19A). The bacterial extract obtained from the fresh samples effectively restored the sorption capacity of I^- in all layers (Figure 19B) and 30 % - 100 % of the sorption was restored after bacterial extract addition depending on the sample layer. In the gyttja layer the values were actually twice as high after the addition of microbial extract to the sterilized sample compared to unsterilized sample.

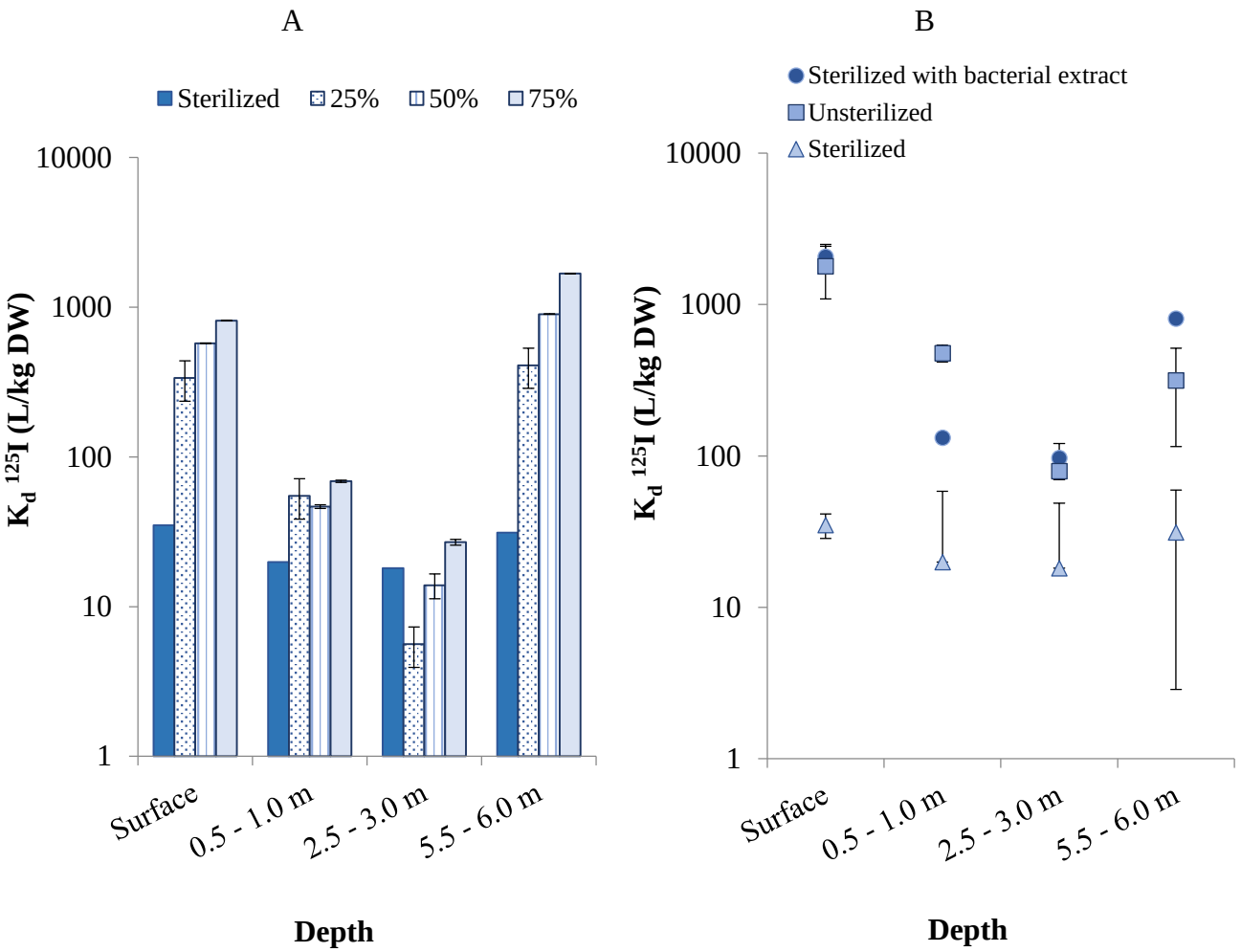


Figure 19. A) The effect of the addition of 0 m-%, 25 m-%, 50 m-% and 75 m-% of fresh peat onto the sterilized peat. B) The effect of the microbial extract addition to the sterilized peat. All values represent the geometric means of duplicate analyses and uncertainty bars indicate the geometric standard deviation. The absence of bars indicates that the uncertainty is smaller than the symbol.

8.6. Characterization of bacterial isolates (Manuscript II)

Of the bacterial isolates obtained from the bog samples, six rapidly growing bacterial strains were chosen for further identification; three Gram-positive and three Gram-negative strains. The bacterial strains (named PS-0-L, V0-1-LW, T5-6-I, K5-6-SY, B6-7-W, and B6-7-CB) originated from surface, 0.5 – 1.0 m, 5.0 – 6.0 m and 6.5 – 7.0 m layers of Lastensuo bog and they were all oxidase-negative and catalase-positive. Based on the 16S rRNA gene sequences, the isolates affiliated with four bacterial groups (Figure 20); *Firmicutes* (B6-7-W and V0-1-LW), *Actinobacteria* (B6-7-CB), *β-proteobacteria* (K5-6-SY) and *γ-proteobacteria* (PS-O-L and T5-6-I). The Actinobacterial isolate B6-7-CB was closely affiliated with the genus *Rhodococcus*, and the *Firmicutes* isolates B6-7-W and V0-1-LW with genus *Paenibacillus* (Figure 20). The β–proteobacterial isolate, on the other hand, was closely similar to the genera *Burkholderia* and *Pandoraea*, while both of the γ-gammaproteobacterial isolates, PS-0-L and T5-6-I, resembled the genus *Pseudomonas*.

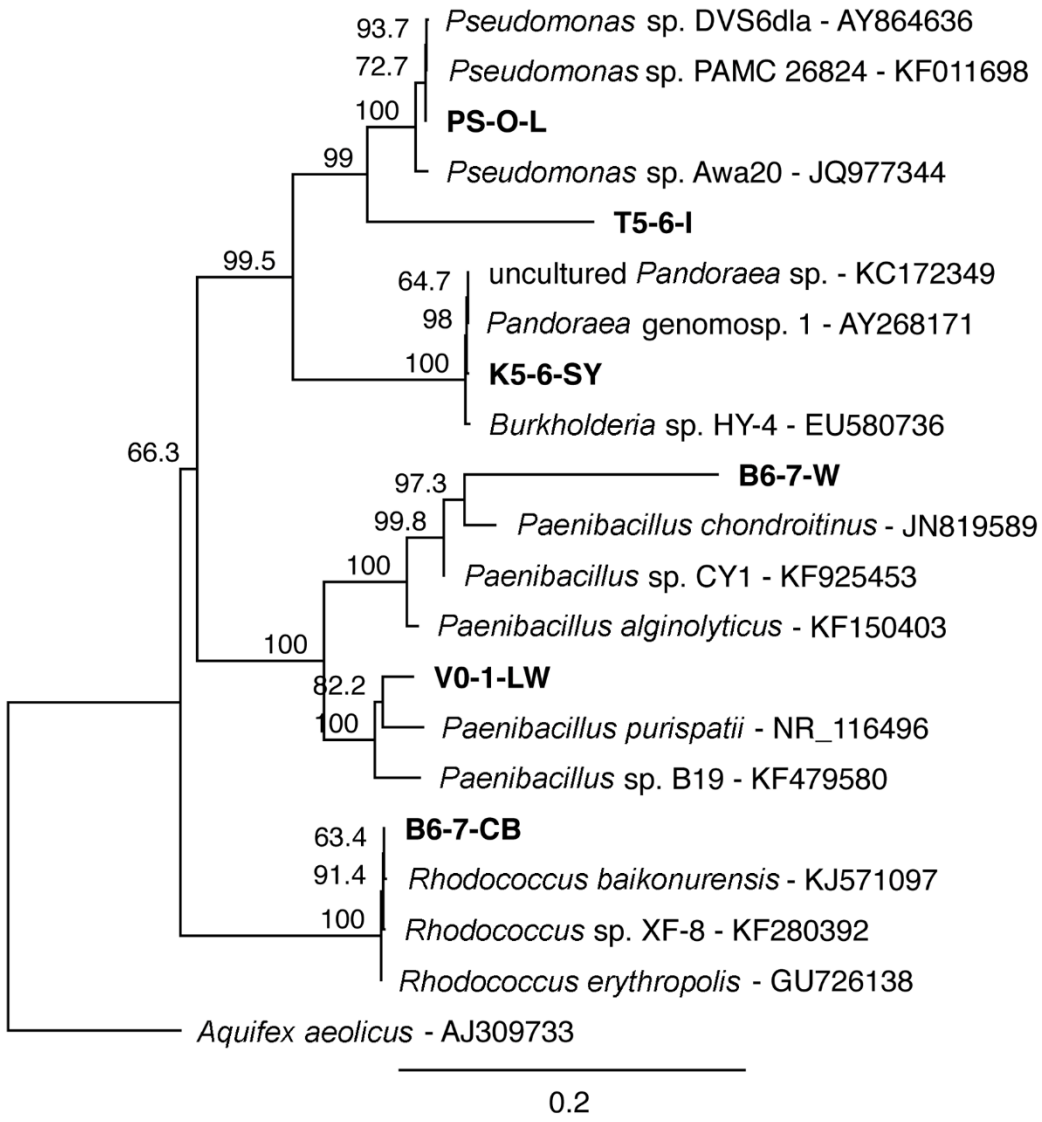


Figure 20. Phylogenetic tree constructed from the 16S rRNA gene sequences of bacterial isolates from Lastensuo bog using the PhyML tool and the JC69 substitution model. The topology of the tree was tested by bootstrap analyses of 100 0 random resamplings.

The three Gram-negative, oxidase negative bacterial strains were further characterized using RapID^{TM} ONE and RapID^{TM} NF Plus systems. Strains *Pseudomonas* T5-6-I and PS-0-L had similar phenotypic characteristics, with the exception of the capability to hydrolyse acrylamide; while *Pseudomonas* T5-6-I could hydrolyse γ-Glutamyl β–naphthylamide and Pyrrolidine-β–naphthylamide, *Pseudomonas* PS-0-L could not. Nevertheless, both strains could utilize nitrate and hydrolysed β-nitrophenyl–phosphoester, proline-β-naphthylamide and N-benzyl-arginine-β-naphthylamide. *Pseudomonas* T5-6-I also hydrolysed γ-glutamyl β–naphthylamide and pyrrolidine-β–naphthylamide. Fatty acids and lipids were hydrolysed by both strains and arginine hydrolysis was variable.

The *Burkholderia* K5-6-SY isolate was stained Gram-negative, similar to *Pseudomonas* T5-6-I and PS-0-L. Urease production and arginine hydrolysis were variable, but ornithine, fatty acids and triglyceride, β-Nitrophenyl–phosphoester, γ-Glutamyl β–naphthylamide and Proline-β-naphthylamide were hydrolysed. Hydrolysis of ρ-Nitrophenyl-n-actyl-β, D-glucosaminide was variable.

8.7. Removal of I^-, SeO_3^{2-} and Cs^+ from nutrient broths and SeO_3^{2-} and Cs^+ from simulated bog water by isolated bacteria (Manuscripts II, III and IV)

All studied bacteria were found to remove I^-, SeO_3^{2-} and Cs^+ from the nutrient broth solution depending on the nutrient broth (A, B, C or D) used, incubation time and temperature. For I^- and Cs^+ low bioremoval was observed with average K_d values around 100 L/kg DW, with an exception of *Paenibacillus* sp. W0-1-LW, for which particularly high bioremoval of I^- (K_d > 1000000 L/kg DW) was observed in one of the used nutrient broths (D). For SeO_3^{2-} significantly higher average bioremoval, compared to that found for I^- and Cs^+ was observed, with an average K_d value of 2900 L/kg DW.

In medium A (1 % Tryptone) and medium B (1 % Yeast extract) the I^- uptake was highest in the *Paenibacillus* B6-7-W (max K_d 270 L/kg DW), *Rhodococcus* sp. B6-7-CB (max K_d 200 L/kg DW) and *Burkholderia* sp. K5-6-SY (max K_d 170 L/kg DW) (Figure 21). For *Paenibacillus* sp. B6-7-W and *Rhodococcus* sp. B6-7-CB, the maximum uptake of I^- was found in medium B, and for *Burkholderia* sp. K5-6-SY in medium A. For *Pseudomonas* sp. PS-0-L, the maximum K_d value of I^- in medium A was 180 L/kg DW and 200 L/kg DW in medium B. For *Paenibacillus* sp. V0-1-LW and *Pseudomonas* sp. T5-6-I the average observed uptake in medium A and medium B was only 10 % from the average uptake of I^- observed for the other studied bacteria in these nutrient broths. Furthermore in medium A and medium B the maximum uptake of I^- by *Paenibacillus* sp. V0-1-LW and *Pseudomonas* sp. T5-6-I was less than 45 % from the maximum uptake observed for *Paenibacillus* sp. B6-7-W, *Rhodococcus* sp. B6-7-CB, *Burkholderia* sp. K5-6-SY and *Pseudomonas* sp. PS-0-L. For *Paenibacillus* sp. V0-1-LW, the maximum K_d values in medium A and medium B were 90 L/kg DW and 60 L/kg DW, respectively. For *Pseudomonas* sp. T5-6-I, the corresponding values were 40 L/kg DW and 90 L/kg DW.

In medium A and medium B, the maximum uptake for *Burkholderia* sp. K5-6-SY and *Pseudomonas* sp. PS-0-L was observed at +37 °C, and for *Pseudomonas* sp. T5-6-I, *Rhodococcus* sp. B6-7-CB and *Paenibacillus* sp. B6-7-W at +20 °C. Similar uptake was found for *Paenibacillus* sp. V0-1-LW at +4 °C and +37 °C.

The effect of glucose on the I^- bioremoval was studied, because for *Flavobacteriaceae* C-21 an iodide uptake mechanism in which glucose is oxidized prior to I^- incorporation into the bacterial cell has been proposed (Amachi et al. 2007). It though seemed that in the bacteria isolated from the nutrient-poor bog, the addition of glucose inhibited the I^- uptake at lower incubation temperatures (+ 4 ºC and + 20 ºC). At + 37 ºC the uptake was however increased in *Pseudomonas* PS-0-L, *Pseudomonas* T5-6-I, *Burkholderia* K5-6-SY and *Rhodococcus* B6-7-CB after glucose addition.

Paenibacillus sp. V0-1-LW was observed to behave quite differently from the other studied bacteria in relation to glucose addition. In medium C, containing glucose, the I^- uptake of *Paenibacillus* sp. V0-1-LW was low (<100 L/kg DW) and a notable increase in the uptake of I^- at all tested temperatures was observed in medium D without glucose, compared to the I^- uptake in all other studied nutrient broths. In medium D, in practice all I^- was removed (99.9 %) from the solution during the one week incubation time, corresponding to a K_d value of >1 000 000 L/kg DW for *Paenibacillus* sp. W0-1-LW in all studied temperatures.

The average SeO_3^{2-} removal from the solution by the studied bacteria was significantly higher than the observed average uptake of I^- or Cs^+. The highest SeO_3^{2-} removal was recorded for the two *Pseudomonas* strains PS-0-L (max K_d 57 000 L/kg DW), T5-6-I (max K_d 6300 L/kg DW) and for *Paenibacillus* sp. B6-7-W (max K_d 11 000 L/kg DW) (Figure 22). When comparing the two nutrient broths, medium A and medium B, on average 7-fold higher rate of SeO_3^{2-} removal by *Pseudomonas* sp. PS-0-L was measured in medium A compared to medium B. For the other bacteria, except of *Paenibacillus* sp. V0-1-LW the uptake in medium A was on average 1.6 times higher than the average uptake found in medium B. For *Paenibacillus* sp. V0-1-LW the average uptake found in medium B was 1.6 times higher than in medium A.

For all studied bacteria the highest K_d values of SeO_3^{2-} for the incubation periods from 1 to 14 days were recorded at + 37 °C and the uptake increased more or less linearly as temperature was increased. The K_d values at + 4 °C were only 2.9 % of the values observed at + 37 °C. The largest difference in the uptake between temperatures of + 4 °C and + 37 °C was found in the *Pseudomonas* sp. PS-0-L strain, for which the uptake at + 4 °C was only 1.5 % of the uptake observed at + 37 °C.

For Cs^+ the highest biosorption was recorded, similarly to I^-, for the *Paenibacillus* sp. V0-1- LW strain, with observed maximum K_d value of 1110 L/kg DW at +37 °C after two weeks of incubation (Figure 23). *Paenibacillus* sp. B6-7-W and *Rhodococcus* sp. B6-7-CB strains exhibited lower sorption and the maximum recorded K_d values for these bacteria were 270 L/kg DW and 85 L/kg DW, respectively. For *Paenibacillus* sp. B6-7-W and *Rhodococcus* sp. B6-7-CB, the maximum sorption was similar to *Paenibacillus* sp. V0-1- LW obtained at +37 °C. For both *Pseudomonas* strains, P-S-0L and T5-6-I the maximum K_d values were recorded after 14 days of incubation at +37 °C. For *Pseudomonas* sp. T5-6-I, the maximum K_d value was, however, only 11% of the value observed for *Pseudomonas* sp. P-S-0L. The maximum K_d values for *Pseudomonas* sp. T5-6-I and *Pseudomonas* sp. PS-0-L were 94 L/kg DW and 840 L/kg DW, respectively.

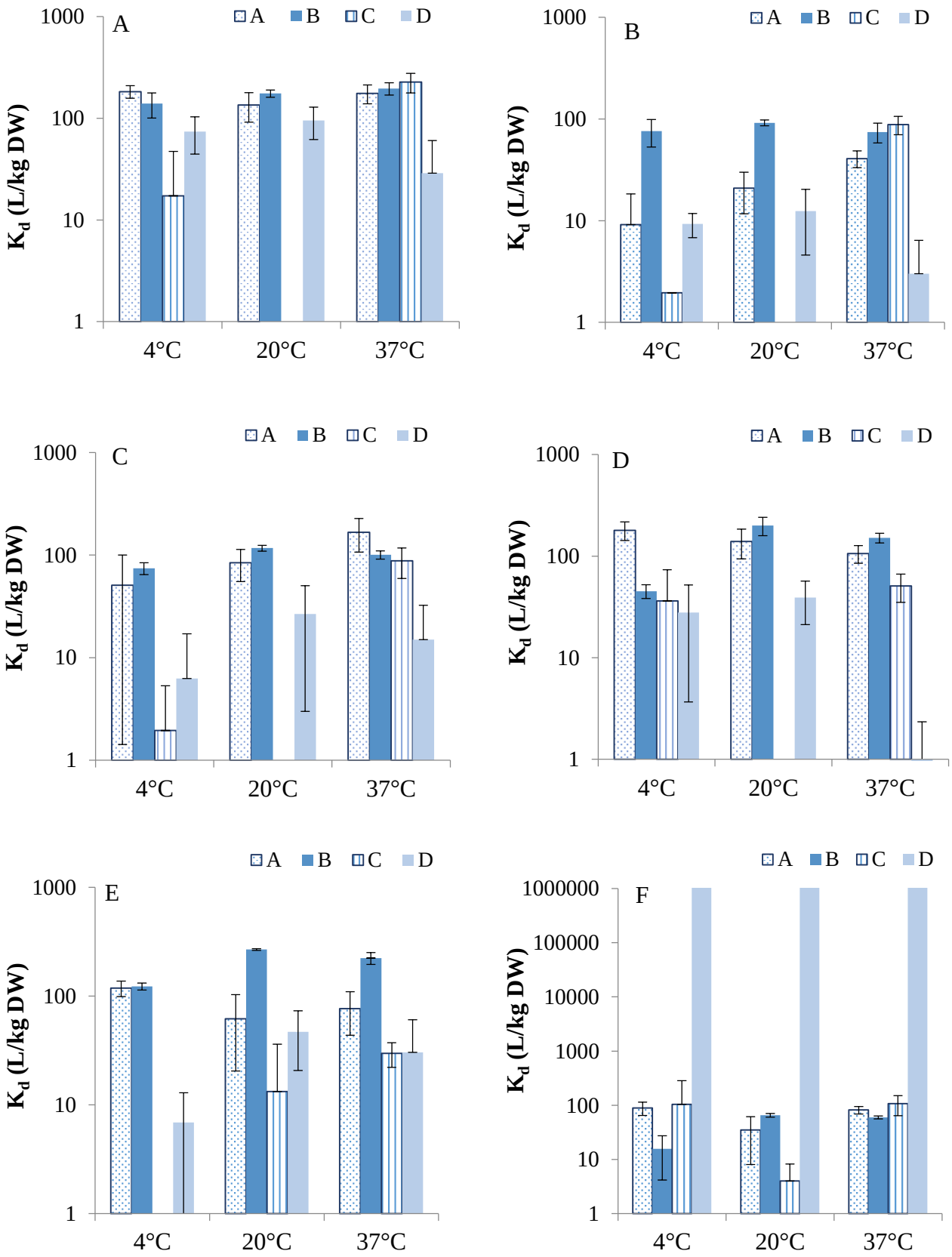


Figure 21. The uptake of I^- by bacteria (L/kg DW) isolated from Lastensuo bog in media A (1% Tryptone), B (1 % Yeast extract), C (0.5 % Peptone+0.25 % Yeast extract+0.1 % Glucose) and D (0.5 % Peptone+0.25 % Yeast extract) at three different incubation temperatures, +4°C, +20°C and +37°C. Incubation time 7 days. (A) Pseudomonas PS-0-L, (B) Pseudomonas T5-6-I, (C) Burkholderia K5-6-SY, (D) Rhodococcus B6-7-CB, (E) Paenibacillus B6-7-W and (F) Paenibacillus V0-1-LW.

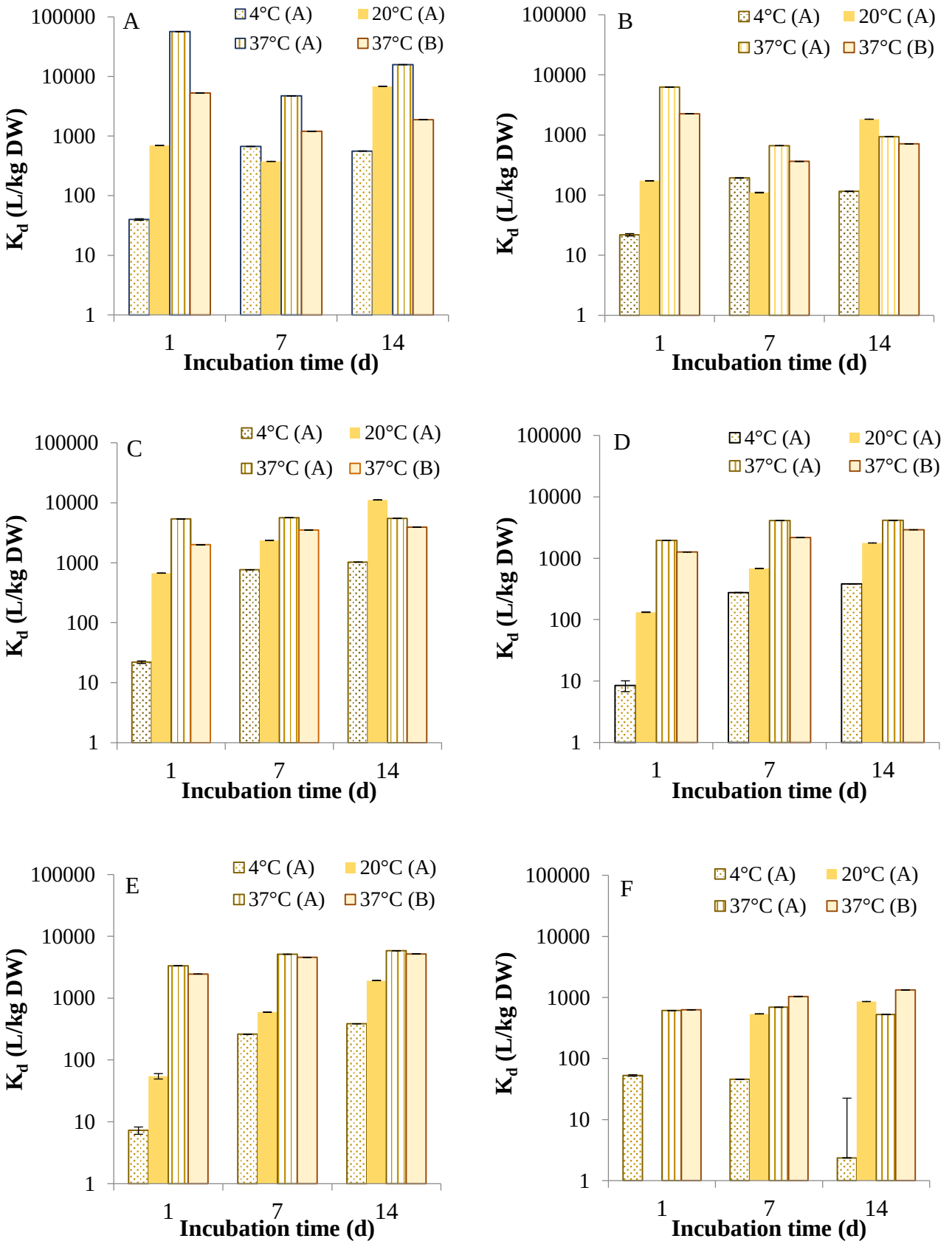


Figure 22. The uptake of SeO_3^{2-} by bacteria (L/kg DW) isolated from Lastensuo bog in media A (1% Tryptone) and B (1 % Yeast extract) at three different incubation times, 1 day, 7 days and 14 days. (A) Pseudomonas PS-0-L, (B) Pseudomonas T5-6-I, (C) Burkholderia K5-6-SY, (D) Rhodococcus B6-7-CB, (E) Paenibacillus B6-7-W and (F) Paenibacillus V0-1-LW.

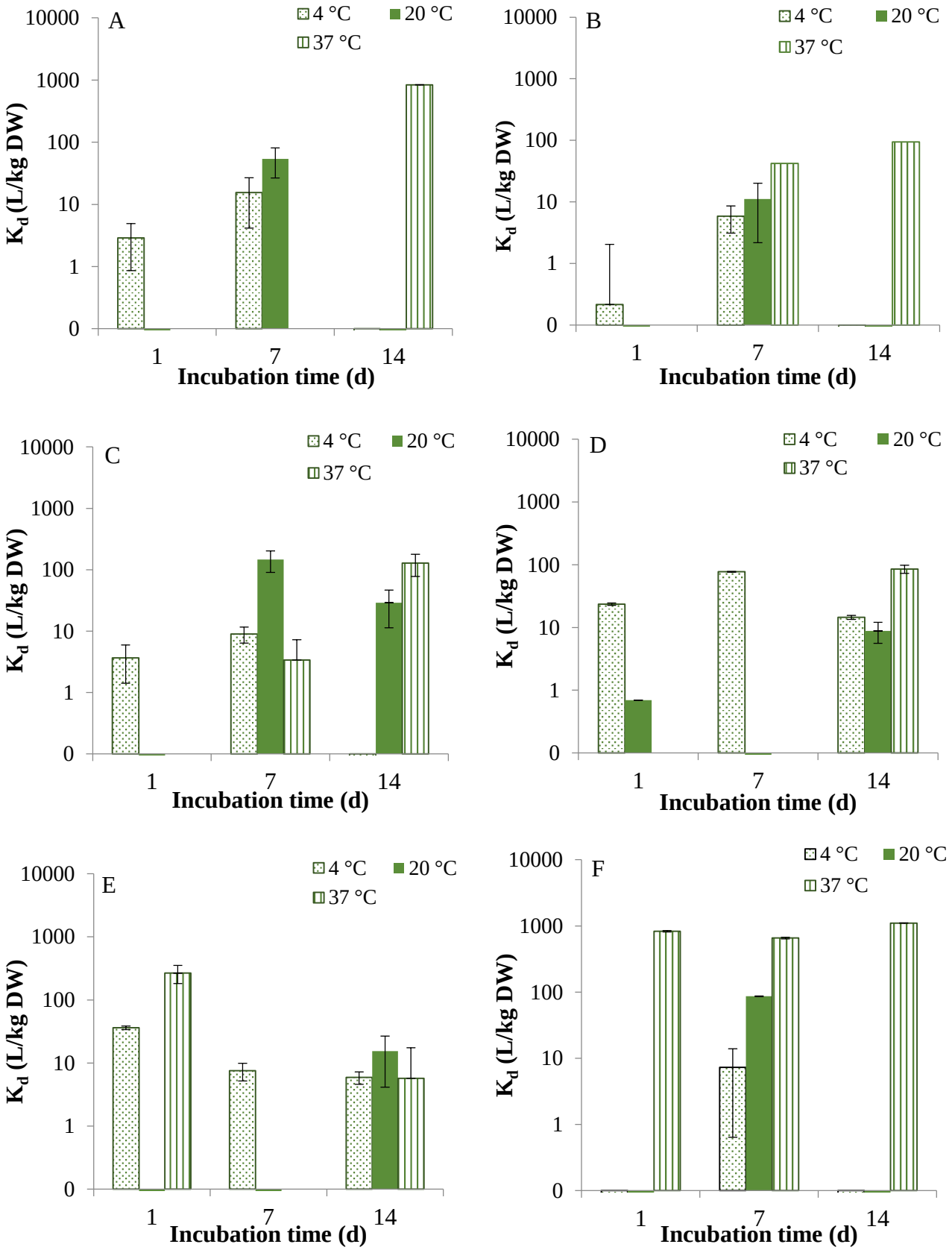


Figure 23. The uptake of Cs^+ by bacteria (L/kg DW) isolated from Lastensuo bog in medium A (1% Tryptone) at three different incubation times, 1 day, 7 days and 14 days. (A) Pseudomonas PS-0-L, (B) Pseudomonas T5-6-I, (C) Burkholderia K5-6-SY, (D) Rhodococcus B6-7-CB, (E) Paenibacillus B6-7-W and (F) Paenibacillus V0-1-LW.

In addition to nutrient broths A, B, C and D sterilized surface moss, peat, gyttja and clay samples in model bog water solution with different bacterial strains was incubated to study the removal of SeO_3^{2-} and Cs^+ from the solution. SeO_3^{2-} and Cs^+ removal from the solution was observed to increase in peat and gyttja layers as isolated bacteria were added to the bog water solution, compared to the removal observed as solely sterilized moss, peat or gyttja was added (Figure 24). In the clay the removal was increased only in the case of SeO_3^{2-}. The increase in SeO_3^{2-} and Cs^+ removal was dependent on the type of bacteria added and for SeO_3^{2-} the most prominent increase, from an average of 3.1 % to an average of 65 %, in the removal in sterilized moss, peat and gyttja was observed as *Pseudomonas* sp. or *Burkholderia* sp. were added to the solution (Figure 24A). For Cs^+ on average a 50 % increase in the removal was observed in the moss, peat and gyttja to which *Pseudomonas* sp. PS-0-L, *Rhodococcus* sp. B6-7-CB or *Paenibacillus* sp. B6-7-W were added, compared to the treatment in which solely sterilized samples were used (Figure 24B). The largest increase in cesium removal, 240 %, was observed in the surface layer as *Rhodococcus* sp. B6-7-CB was added to the sample.

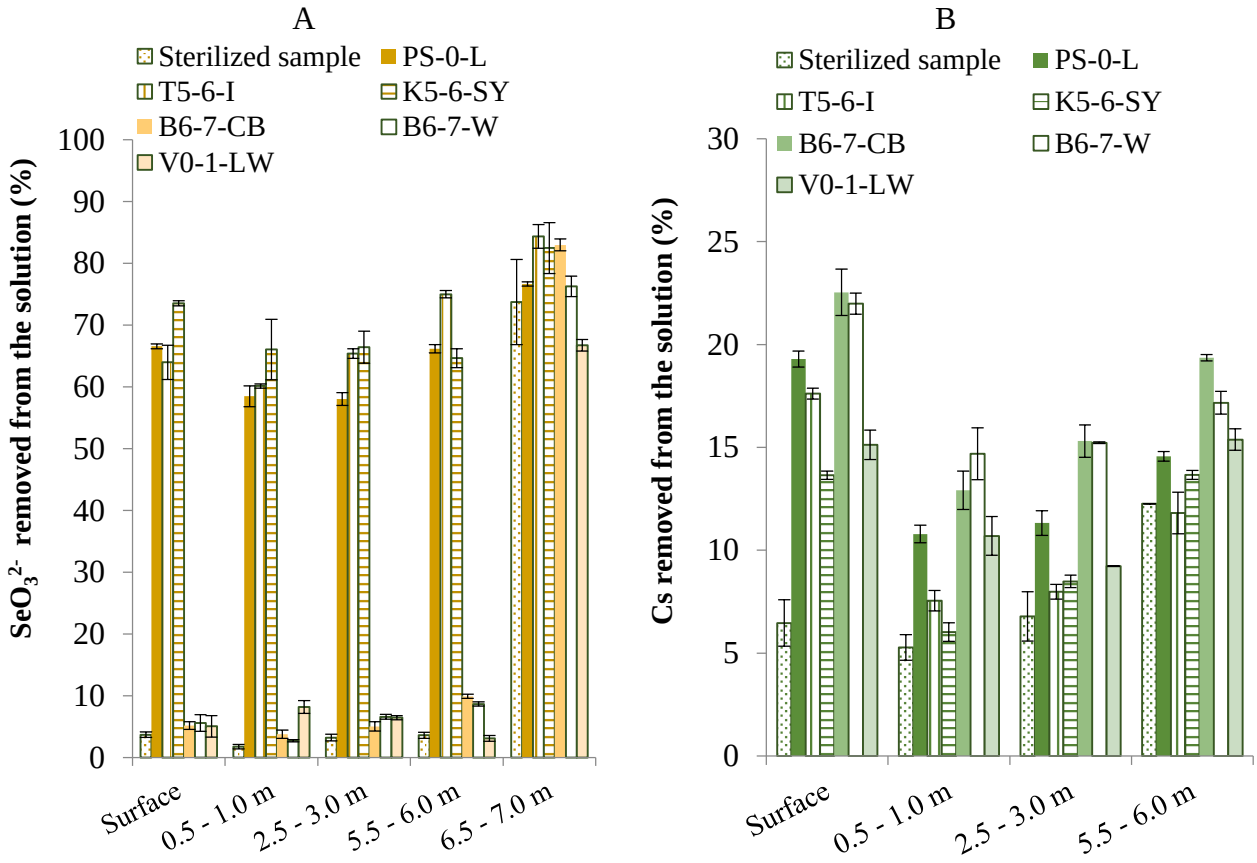


Figure 24. (A) The removal of SeO_3^{2-} (%) from the bog water model solution when 2 % sterilized surface moss, peat, gyttja or clay and different bacterial strains were added to the solution. T = + 20 °C, incubation time seven days. (B) The removal of Cs^+ (%) from the bog water model solution when 2 % sterilized surface moss, peat or gyttja and different bacterial strains were added to the solution. T = + 20 °C, incubation time seven days.

9. Discussion

I^- and SeO_3^{2-} are considered highly mobile in the environment due to their anionic form. I^- mobility is increased especially under anoxic conditions and its retention is typically low on mineral soils. It has been shown in several studies (e.g. Whitehead 1974, Sheppard and Thibault 1992, Sheppard et al. 1995, Assemi and Erten 1994, Fukui et al. 1996, Yoshida et al. 1998, Kaplan 2003) that the organic matter and pH affect the I^- sorption. In the organic peatland with a low pH that was explored in this study I^- retention was expected to be elevated. Selenium possesses multiple oxidations states, which depend on the pH and redox conditions and microbial reduction is one of the major factors influencing the behaviour of selenium oxyanions (SeO_4^{2-} and SeO_3^{2-}). In the organic matter the retention of I^- is assumed to occur between I^- oxidized enzymatically into I_2/HIO and organic molecules (Xu et al. 2013). SeO_3^{2-} retention on the other hand is assumed to involve bacterial SeO_3^{2-} reduction using organic substrate molecules (Dungan and Frankenberger 1999). However, direct association of both anions with protonated nitrogen-containing groups ($-NH_3^+$) of organic matter cannot be completely ruled out. In addition in the clay layer (6.5 – 7.0 m) with low organic matter content, the retention of I^- and SeO_3^- is assumed to occur partly via inner- and outer-sphere complexation involving OH groups found on the surfaces of silicate and oxide minerals. The formation of selenide (Se^{2-}) and its interaction with divalent iron is also possible.

Cs^+ is known to strongly sorb on the frayed edge sites (FES) of clay and mica minerals, resulting in low mobility and low bioavailability in soils having a high clay and mica mineral content (e.g. Saengkul et al. 2013, Chang et al. 1993, Staunton et al. 2002, Lieser and Steinkopff 1989, Gutierrez and Fuentes 1996, Houmani et al. 2012), but in organic matter such as peat Cs^+ retention is known to be significantly lower compared to that observed in clay and mica minerals (e.g. Saengkul et al. 2013, Chang et al. 1993, Staunton et al. 2002). In SOM, the sorption of Cs^+ is affected by the pH of the solution and a decrease in pH typically reduces the sorption of cations in SOM. This is because the surface functional groups, such as carboxylic groups, become protonated, preventing the sorption of positively charged cations on these groups through ion exchange. In the bog environment with low pH the retention of Cs^+ by the components of SOM was therefore expected to be low.

9.1. Sorption kinetics and sorption isotherm models

The decrease in the temperature from +20 °C to +4 °C reduced the sorption rates of both I^- and SeO_3^{2-}. Characteristically an increase in the sorption rate with increasing temperature is expected, as the rate of many organic reactions typically increases with temperature. This is because of the greater average kinetic energy of the molecules in higher temperatures. The rate of enzyme-catalyzed reactions also increases with temperature due to the increasing kinetic energy of both enzyme and substrate molecules and especially for bacteria, the dependence of enzyme activity on temperature is significant (Becker et al. 2003). Several steps are expected to be involved in the retention of I^- and SeO_3^{2-} in peat. These steps include the transport of I^- and SeO_3^{2-} from the aqueous phase to the surfaces of organic matter and bacteria, the presumed reduction reactions of SeO_3^{2-} and oxidation reactions of I^- and

possible transport of reduced/oxidized species into the cell interior. Even if a proportion of I^- or SeO_3^{2-} retention would occur via direct association into the organic matter, an increase in the sorption rate, resulting from the increase in temperature, would be expected, because of the greater kinetic energy of the molecules. The direct association of I^- and SeO_3^{2-} into the organic matter cannot therefore be completely ruled out. In any case the retention of I^- and SeO_3^{2-} into the organic matter occurs via various steps and several phases can act as rate limiting steps.

The kinetics of sorption can be described and predicted using different models. Elovich and pseudo-second-order (PSO) kinetic models with K_d (Shetaya 2011) were used in this study for the I^- and SeO_3^{2-} and the Elovich model was found to better describe the kinetics of I^- and SeO_3^{2-} retention, compared to the PSO model. Even though the Elovich model does not provide any mechanistic explanation for the sorption behaviour, it has been used to predict the sorption kinetics in highly heterogeneous systems and to describe a number of reaction mechanisms, including the activation of catalytic surfaces and surface diffusion (Ho and McKay 2002, Sparks 2003).

The Freundlich sorption model was used to describe the sorption of Cs^+ and the Freundlich plots had high linearity in the surface moss, peat and gyttja layers. In these layers the N values were between 1.0 and 1.1. In the clay layer some deviation from the isotherm was observed and the N value was 1.4. The Freundlich model has been used for various applications such as activated carbon, silica, clays and polymers (e.g. McKay and Al-Duri 1991, Narges 2012, Vimonsesa et al. 2009, Umpleby et al. 2001) and it usually best fits the experimental data at low concentrations. Typically the observed isotherm starts to deviate from the Freundlich isotherm as the saturation point is approached (Umpleby et al. 2001), or as sorption on less selective sorption sites occurs.

9.2. The biotic effects on the sorption behaviour of I^-, SeO_3^{2-} and Cs^+

9.2.1. The effect of microbiota in the bog

In the present study the sorption of both studied anions, I^- and SeO_3^{2-}, was found significantly lower in the sterilized samples of surface moss, peat and gyttja compared to the unsterilized samples. For these ions on average only 2 – 3 % of the sorption observed in the unsterilized samples was recorded for the sterilized ones in the organic layers. The incubation of sterilized samples with fresh peat and microbial extract demonstrated that the recolonization of sterilized samples could restore the sorption capacity of I^- in surface moss, peat and gyttja samples, and hence it was concluded that the decrease caused by sterilization was due to the destruction of micro-organisms and/or their products rather than the alteration of organic matter. For Cs^+ the effect of sterilization was less prominent and its sorption was on average 38 % lower in sterilized samples compared to the unsterilized samples, but statistical difference between sterilized and unsterilized samples could not be proven.

The results obtained for the sterilized surface moss, peat and gyttja samples indicate that microbiota play an important role in the immobilization of both I^- and SeO_3^{2-} into the organic matter. Furthermore in the deepest clay layer, with the lowest abundance of organic matter, the sterilization of the samples decreased the sorption of SeO_3^{2-} by 80 %, indicating that microbiota is not only necessary for the

retention of SeO_3^{2-} in the organic layers, but also in the mineral fraction of the bog. Similarly for I^- the sorption in sterilized clay samples was significantly lower than the sorption of I^- observed in the unsterilized samples. It is assumed that in the clay layer, some proportion of the I^- sorption occurs between oxidized I^- species and organic molecules present in the clay layer or as I^- sorption on protonated nitrogen containing groups through electrostatic interactions without oxidation, in addition to the sorption on the silicate and oxide minerals through outer-sphere complexation. The assumption of I^- sorption via enzymatically oxidized I_2/HIO is supported by the observation that in the sterilized samples of the clay layer, the sorption of I^- was only on average 9% of the sorption of I^- on the unsterilized samples of this layer. If merely the abiotic sorption of I^- on the silicate and oxide minerals or protonated nitrogen-containing groups of organic matter would occur, one would expect no effect of sterilization on the sorption of I^-. This hypothesis is also supported by the fact that the sorption of I^- in the clay layer was found to be at the same very low level in both sterilized samples and samples incubated in anoxic conditions. It is possible that in addition to bacteria, certain eukaryotic micro-organisms such as filamentous fungi contribute to I^- oxidation (Amachi et al. 2005), enabling the further incorporation into the organic matter.

9.2.2. The effect of bacteria in the bog

The sterilization of samples affects directly the bacteria associated with the sorption behaviour of both I^- and SeO_3^{2-}. If a large number of bacteria increased the sorption, the bacterial numbers in the samples with high K_d values are expected to be greater than that found in the samples with low K_d values. The bacterial counts were determined from the sorption experiment samples of I^- using PCA and the bacterial counts were found to correlate positively with the I^- K_d values at +4 °C. Unfortunately, for the data from the incubations at +20 °C correlation analysis was not possible because of very high, uncountable bacterial numbers found on the PCA plates at this temperature. This, nevertheless, shows that in the samples incubated at +20 °C, the number of bacteria was higher than in those incubated at +4 °C. It is however worth noticing that the bacterial numbers obtained using PCA represent only the number of bacterial cells able to grow in this media under laboratory conditions. Majority of the bacteria found in a nutrient-poor bog such as Lastensuo are unlike to be cultivable on this nutrient-rich bacterial growth medium. The bacterial counts obtained using PCA are, however, approximate and provide more evidence to support the assumption that the bacteria found in the bog enable the sorption of I^- in this environment. A plate is deemed uncountable if it contains more than 500 colonies. Therefore, as the smallest dilution used in this study was 10^{-5}, it can be estimated that at least 5×10^8 colony forming units were obtained from the incubations at +20 °C. The original number of bacteria g^{-1} DW sample was estimated to be approximately 10^{10} (Tsitko et al. 2014).

The surface moss layer of Lastensuo bog had the highest abundance of *Proteobacteria*, especially *α-proteobacteria* and their relative abundance significantly decreased with depth (Tsitko et al. 2014). Many of these α-proteobacterial species have been shown to be able to oxidize I^- to I_2 (Arakawa et al. 2012). In addition many proteobacterial species, such as *Chromatium vinosum, Desulfovibrio desulfuricans, Sulfospirillum barnesii* and *Ralstonia metallidurans* are known to reduce selenite

/selenate into elemental Se^0 both in anoxic (e.g. Oremland et al. 1989, Oremland et al. 2004, Oremland et al. 2014, Nelson et al. 1996) and oxic (e.g. Fujita et al. 1997, Sarret et al. 2005, Lortie et al. 1992) conditions. In the clay layer of the bog *Firmicutes*, bacteria which also include many significant selenite reducing bacteria, were found (Tsitko et al. 2014). Such *Firmicutes* include e.g. *Selenihalanaerobacter shriftii, Paenibacillus selenitireducens* and *Bacillus subtilis* (Oremland et al. 2004, Combs et al. 1996). Presumably in this layer a major proportion of SeO_3^{2-} is reduced microbiologically into Se^0 and part of the selenite is sorbed on the OH groups of silicate and oxide minerals through surface complexation without reduction. A small proportion of SeO_3^{2-} reduction by sulfide is also possible. Abiotic processes are however expected to be quite low based on the observation that sterilization of the clay samples reduced SeO_3^{2-} removal significantly.

To further clarify the importance of bacteria on the sorption and retention of I^-, SeO_3^{2-} and Cs^+, bacteria were isolated from the bog samples and used in the batch sorption experiments. The bacteria captured in pure cultures represented a minority of the bacterial community in the bog previously described by HTP sequencing (Tsitko et al. 2014). However, all isolated bacteria were able to remove all studied ions, I^-, SeO_3^{2-} and Cs^+ from the solution, depending on the incubation conditions. This indicates that I^-, SeO_3^{2-} and Cs^+ uptake is a common trait for bacteria in this environment. From the three studied ions, the highest bioremoval was observed for SeO_3^{2-}.

For I^- the highest uptake were shown for the Gram-positive *Paenibacillus* strains B6-7-W and V0-1-LW and for *Rhodococcus* B6-7-CB, which are common soil dwelling bacteria. *Paenibacillus* are facultative anaerobic bacteria which have been previously isolated e.g. from forest soil, fen peat soil and spacecraft assembly clean room *(*Zhao and Li 2008, Lepleux et al. 2012, Behrendt et al. 2010*). Rhodococcus* are aerobic Gram-positive, non-sporulating bacteria with high G-C content and they have been found in a broad range of environments, including soil, water, forest soil and eukaryotic cells (Servin et al. 2008, van der Geize and Dijkhuizen 2004, Dong et al. 2010). *Rhodococcus* have broad catabolic diversity and are of biotechnological importance due to their ability to synthesize several products such as surfactants, flocculants, amides and polymers as well as bioactive steroids, acrylamide and acrylic acid (Bell et al. 1998).

For SeO_3^{2-} the highest bioremoval was observed in the two *Pseudomonas* sp. strains PS-0-L and T5-6-I, belonging to the proteobacterial phylum, members of which are known to reduce SeO_3^{2-} into elemental Se^0 (e.g. Nelson et al. 1996, Oremland et al. 2004). In addition high removal of SeO_3^{2-} was observed in *Burkholderia* sp. K5-6-SY, belonging to the proteobacterial phylum, and for one of the *Paenibacillus* sp. strains, belonging to the phylum Firmicutes, which as stated above are also known to reduce selenite. Significant removal of SeO_3^{2-} was also recorded for *Rhodococcus* sp. belonging to phylum Actinobacteria. Actinobacteria exhibit a wide spectrum of morphologies and highly variable physiological and metabolic properties and comprise one of the largest bacterial phyla widely found in aquatic and terrestrial soils (Servin et al. 2008, Ventura et al. 2007).

Only little is known about the SeO_3^{2-} metabolism among bacteria, but it has been suggested that its uptake is via the sulphate ABC transporter complex described in the proteobacterium *E. coli*. This complex is composed of two CysA ATP-binding proteins, two transmembrane proteins (CysT and CysW) and a periplasmic sulphate binding protein CysP (Rosen and Liu 2009, Turner et al. 1998) (Figure 10). However, an alternative, yet unidentified carrier is also supposed to exist for SeO_3^{2},

because the repression of sulphate permease expression does not completely inhibit the SeO_3^{2-} uptake in *E. coli* (Turner et al. 1998). Inside the cell SeO_3^{2-} is reduced and at least partially incorporated into amino acids as selenomethionine and selenocysteine (Turner et al. 1998).

In addition to I^- and SeO_3^{2-}, all isolated bacterial strains also removed Cs^+ from the solution. As in the case of I^-, the highest removal of Cs^+ was recorded for one of the *Paenibacillus* sp. strains, V0-1-LW. Biosorption higher than the average was additionally recorded for *Pseudomonas* sp. PS-0-L. This differs from the results reported by Luk'yanova et al. (2008) for *P. fluorescens* C50-1, *P. stutzeri* 116-1-1 and *P. putida* C49-2 isolated from deep groundwater, which did not sorb cesium. *Pseudomonas* are some of the most abundant bacterial genera globally and have been found in diverse habitats, such as water, humans, animals, plants, fungi, clouds and artic and desert soils (Peix et al., 2009).

As it was observed that the different nutrient broths affected the removal of SeO_3^{2-} and Cs^+ from the solution by all studied bacteria, sterilized bog samples and sterile bog water model solution was used as a nutrient source. This was done to study the removal of SeO_3^{2-} and Cs^+ from solution in nutrient conditions closer resembling the conditions found in the bog. In the case of Cs^+ as well as SeO_3^{2-} bacteria isolated from the bog added to sterilized surface moss, peat and gyttja samples, increased the removal of these ions from the solution, especially SeO_3^{2-}. For both Cs^+ and SeO_3^{2-} the increase in the removal was found statistically significant. Similarly to different nutrient broths (1 % Tryptone and 1 % Yeast extract), SeO_3^{2-} was effectively removed from the solution by *Pseudomas* sp. strains and *Burkholderia* sp. strain when surface moss, peat or gyttja was used as a nutrient source. For Cs^+ an especially clear increase in the removal of on average 50% was observed when *Pseudomonas* sp. PS-0-L, *Rhodococcus* sp. B6-7-CB or *Paenibacillus* sp. B6-7-W were added to sterilized surface moss, peat and gyttja samples. These results demonstrate that bacteria can affect, not only the retention of anionic I^- and SeO_3^{2-} in the bog environment, but also, at least marginally, the sorption behaviour of Cs^+. For I^- it was estimated that the direct uptake of I^- by bacterial cells accounted for approximately 0.1 % of the total sorption in the surface layer, 0.2 % in the subsurface, middle and gyttja layers (0.5 – 6.0 m), and 0.3 % in the clay layer (6.5 – 7.0 m) of the bog (manuscript II). However the indirect bacterial effect through oxidation of I^- into I_2/HIO prior to its incorporation into the organic matter is significant, which is supported by the fact that the sterilization decreased the sorption of I^- drastically.

9.2.3. The effect of surface moss on the sorption of Cs^+

The batch K_d values of Cs^+ were about one order of magnitude lower than the *in situ* K_d values obtained from the 1 M ammonium acetate extraction, and in the surface moss layer the difference between batch values and *in situ* values was even higher. In the surface moss, the experimental batch K_d value of Cs^+ was only 1.1% of the value recorded for the *in situ* approach. In the experimental batch K_d determinations the ^{133}Cs concentration/sample (carrier concentrations in the ^{134}Cs tracer) was 8×10^{-10} M, which corresponds to the ^{133}Cs concentrations found in the bog water (1.5×10^{-10} M - 7.5×10^{-10} M). At this concentration range the cesium concentrations are not expected to affect the K_d value, especially as the cesium concentrations in the both approaches are close to each other. 1 M

ammonium acetate is commonly used to extract the exchangeable cations, but it cannot distinguish whether the ^{133}Cs came from the exchange sites of organic matter, or the minor mineral components possibly found on the surfaces of organic matter. It is uncertain if the *in situ* values obtained using relatively robust 1 M ammonium acetate extraction method can be used to assess the radiocesium sorption in the long term safety analysis of spent nuclear fuel because of the clear difference between this approach and the batch approach and therefore further study on the possible extraction methods is needed. However some useful information about the processes affecting the sorption of cesium in the bog can be deduced from the differences between the batch and *in situ* approach. In surface moss, active plant uptake of Cs^+ is possible under *in situ* conditions. Plants readily sorb Cs^+ from the solution, most probably through the K^+ uptake system (Zhu and Smolders 2000), and mosses in particular are known to accumulate cesium (e.g. Dołhańczuk-Śródka et al. 2011, Nimis 1996, Dragović et al. 2002). Only the exchangeable fractions of Cs^+ are extracted by ammonium acetate extraction, used in this study (Gogo et al. 2010) and the Cs^+ incorporated into the moss should not therefore be extracted. However, mosses are known to sequester cations on exchange sites and cations are held on their surfaces until they are moved into the plant (Wells and Brown 1990, Bates 1997). *Sphagnum* moss, in particular, is known to accumulate cations through passive cation exchange (Wojtun 1994). Due to the interactions between roots and soil, the concentrations of exchangeable cations are higher both in the inner and outer rhizosphere of vascular plants compared to the bulk soil (e.g. Collignon et al. 2011, Schöttelndreier and Falkengren-Grerup 1999). Unlike vascular plants, mosses take up nutrients over their entire surface. Even though mosses do not have actual roots, they have analogous single-celled structures called rhizoids, and the movement of ions through cation exchange and the proton pump of mosses most likely has a similar influence on the rhizoidosphere as vascular plants have on the rhizosphere (Raven et al. 1998). It is likely that these interactions are not captured in a batch experiment. Therefore, significantly lower K_d values might result in the surface moss layer than would take place if plant uptake of Cs^+ and interactions between rhizoids of the moss and bog water were taken into account.

9.3. The effect of anoxic conditions on the sorption of I^- and SeO_3^{2-}

I^- sorption was found notably lower in anoxic conditions compared to oxic conditions in all studied layers. For SeO_3^{2-} the effect of anoxic conditions was less clear. Under anoxic conditions the mean K_d values of SeO_3^{2-} found for the longer incubation periods (up to 84 days) closely corresponded to the values observed under oxic conditions especially in the gyttja (5.5 – 6.0 m) and clay (6.5 – 7.0 m) layers and in the subsurface peat layers from 0.5 to 3.0 m the K_d values were somewhat higher in the samples incubated under oxic conditions, compared to the samples incubated under anoxic conditions. These results together with those obtained for the sterilized samples support the theory that under aerobic conditions SeO_3^{2-} is microbiologically reduced (e.g. *R. metallidurans* CH34) in the presence of organic substrates (Stolz and Oremland 1999). If SeO_3^{2-} would not be reduced under oxic conditions, it would be expected that the K_d values obtained under oxic conditions would differ clearly from the values obtained under anoxic conditions. This is because SeO_3^{2-} is expected, based on the pH and Eh values obtained from the batch sorption experiments, to be in reduced form under anoxic conditions. Several bacteria (e.g. *S. oneidensis, S. barnesii, B. selenitireducens, S. shriftii*) are

known to reduce SeO_3^{2-} under anoxic conditions. In addition abiotic reduction may also take place, as Fe(II) is known to reduce SeO_3^{2-} under acidic and reducing conditions (Charlet et al. 2007) and also the reduction with sulfide is possible (Pettine et al. 2012). Secondly, if merely the abiotic reduction of SeO_3^{2-} would occur, the sterilization of the samples would not be expected to inhibit the retention of SeO_3^{2-} as was the case in this study.

The mechanism of I^- sorption onto the organic matter under anoxic conditions is not completely clear, but low-level sorption on protonated nitrogen groups found in the organic matter can be expected. The probable explanation for the low I^- sorption level in anoxic conditions, corresponding to the sorption found in sterilized samples, is the absence of microbially catalysed I^- oxidation to I_2/HIO, and therefore decreased sorption.

9.4. The effect of pH on the sorption of I^-, SeO_3^{2-} and Cs^+

pH was found to affect the sorption of all studied ions, I^-, SeO_3^{2-} and Cs^+. The surface charge of organic matter is dependent on the pH, and the sorption of cations typically decreases as the pH value decreases. For the anions the effect is opposite. Peat components have a broad spectrum of pKa values and their surface chemistry includes the dissociation and protonation reactions of acidic carboxyl groups, alcoholic and phenolic –OH groups and amino groups (e.g. Andreasson et al. 1988, Strelko and Malik, 2002; Corapcioglu and Huang, 1987, Tan 2003). The majority of the complexing sites are found in carboxyl groups (Sumner 2000). The carboxylic acids dissociate at higher pH values, and for the carboxylic acids found in soil organic matter pKa values around 3–4 have been reported (Lal, 2006, Tan, 2014). Dissociation increases the net negative charge, and cations, like Cs^+ can bind to these negatively charged exchange sites in an exchangeable form. In our study, the sorption of Cs^+ was found to considerably increase between pH 2 and pH 7 in the upper peat (0.5–3.0 m) and gyttja (5.5–6.0 m) layers, having a high proportion of organic matter, and the maximum sorption of cesium was detected between pH ~7.0–9.5. If a pKa value around 4 for the carboxylic groups is assumed, 99% of the functional carboxylic groups would be protonated at pH 2, preventing the sorption of cationic Cs^+ on these groups. As pH is increased, the proportion of dissociation increases, and at pH 7 practically all carboxylic groups would be in a dissociated form. Cs^+ bound to these pH-dependent cation exchange sites is readily leached as pH decreases. It is worth noting that in bog environment, the fluctuations of pH are common, and the daily variations in pH can be from 0.5 to 1.0 pH units (Tahvanainen and Tuomaala 2003). Typically the bog water pH decreases at night due to the accumulation of carbon dioxide, while in the daytime pH increases as plants use carbon dioxide during photosynthesis (Tahvanainen 2005). The decrease in the cesium K_d values at higher pH range (> 8) most probably results from the dissolution of organic matter. The solubility of organic matter is dramatically increased at pH values above 7 – 8, which results in a high content of dissolved organic matter (DOC) (Fanum 2014). On the other hand under highly acidic conditions organic matter can be mobilized as organic colloids (Fanum 2014). The concentrations of DOC are not available from our pH study samples. However the DOC concentrations in the bog water samples (Table 1) were found to increase with increasing bog water pH.

Nitrogen cycling plays an important role in the biogeochemistry of peatlands and ombrotrophic bogs are known to act as sinks for atmospheric ammonia (Urban and Eisenreich 1988, Sheppard et al. 2013). Although the NH_4^+ concentrations, selectivity coefficients (Cs^+/K^+, Cs^+/NH_4^+) and the nitrogen cycling in the bog were beyond the study objectives of this thesis, it is worth noting that NH_4^+ can affect both the pH and the sorption of Cs^+ and nitrogen cycling has been identified as a major energy generating metabolic pathway in the bacterial community of the Lastensuo bog (Tsitko et al., 2014). NH_4^+ and K^+ compete with Cs^+ for the sorption sites, as both have similar hydrated radius as Cs^+ (Smith and Comans 1996). The K_d values of ^{137}Cs have been shown to be inversely proportional to the NH_4^+ concentrations of soil solution (Smith and Comans 1996) and plant uptake of NH_4^+ e.g. by bryophytes found in the bog can result in a net pH decrease of the bog water solution (Morel and Hering 1993). pH is also affected by the microbiologically mediated nitrification processes in which NH_4^+ is changed into NO_2^- concurrently decreasing pH (Morel and Hering 1993). It has also been suggested that low NO_3^- concentrations in the soil solution could be connected to high NH_4^+ concentrations, due to microbial dissimilatory nitrate reduction to ammonia (Sheppard et al. 2013, Giblin et al. 2013). In present study the bog water NO_3^- concentrations were found lowest in the surface of the bog (0.1 mg/L). In the peat and gyttja layers the bog water NO_3^- concentrations were nearly constant at on average 4.0 mg/L.

For the anions I^- and SeO_3^{2-} the maximum sorption in the surface moss, peat and gyttja layers (and for SeO_3^{2-} also in the clay layer) was observed between pH ~ 3.0 – 5.0. For both I^- and SeO_3^{2-} an increase was observed between pH 2 and 4, where after the K_d values of I^- decreased noticeably as pH was increased. Fukui et al. (1996) have reported that in the pH range of 3.2–10.6, the uptake of I^- in soil decreased as a function of increasing pH and that at pH 3.2 I^- was converted into I_2. At the lower pH, the conversion of I^- into I_2 (and subsequently to HIO) might result from the more efficient enzymatic oxidation of I^-, utilizing microbial peroxidases present in organic matter. The microbial peroxidases correlated positively with I^- K_d values at + 20 °C in present study and for the peroxidases optimum pH values, depending on the type of the peroxidase, have been reported to be around pH 3–6 (e.g. Loprasert et al. 1988, Hochman and Goldberg 1991, Mizobutsi et al. 2010, Kalyani et al. 2011). This corresponds to the pH range in which the highest I^- sorption was observed in the surface moss, peat and gyttja samples of this study. In addition, nitrogen-containing groups (e.g. amino groups) found in organic matter are protonated under acidic conditions and I^- can sorb onto the resulting positively charged groups through electrostatic interactions.

For SeO_3^{2-} the K_d values remained relatively constant between pH 3 and pH 6.5 in all layers, after which the K_d values decreased strongly. This correspond to the earlier studies, for instance by Su and Suarez (2000), who reported SeO_3^{2-} sorption to decrease as a function of increasing pH at the pH range from 5 to 9 in Dakota soils. If the microbially induced reduction of SeO_3^{2-} is expected to participate in the removal of SeO_3^{2-} from the solution, the higher removal of SeO_3^{2-} at pH below 6.5 might result, similarly to oxidation of I^-, from the more efficient enzymatic conversion of SeO_3^{2-} into Se^0 at a lower pH. Even though the enzymes involved with the reduction of SeO_3^{2-} are poorly known (Hunter 2014), nitrite reductases, fumarate reductase FccA and OYE enzyme (Old Yellow Enzyme, NADPH oxidoreductase) have been suggested to be potentially involved in the selenite reduction (e.g. DeMoll-Decker and Macy 1993, Li et al. 2014, Hunter 2014). The optimum pH values reported for nitrite reductases are around pH 5–7 (e.g. Iwasaki and Matsubara 1972, Ellis et al. 2001, Sawada

et al. 1978, Wang et al. 2013) and for fumarate reductase FCC3 from *Shewanella frigidimarina* at pH below 6 (Pankhurst et al. 2002). These optimum - pH values correspond to the pH range detected in present study after which the K_d values of SeO_3^{2-} were observed to decrease in all studied layers. In addition similarly to I^-, SeO_3^{2-} can sorb onto the positively charged nitrogen-containing groups found in organic matter through electrostatic interactions.

In the clay layer the sorption of both I^- and SeO_3^{2-} is assumed to involve OH groups in silicate and oxide minerals found in this layer. The surface hydroxyl groups are protonated at a low pH and unoxidized I^- ions form outer-sphere and SeO_3^{2-} inner-sphere complexes with these groups. As the pH is increased, the protonated groups are dissociated, first forming a neutral and then a negative surface, which results in a lower anion exchange capacity at higher pH values, but increasing the cation exchange capacity. For Cs^+, an increase in the sorption with increasing pH from 2 to 7 was similarly to the organic layers observed also in the clay layer, although the increase was less steep. Between pH 2 and pH 4, however, a considerable 1.7-fold increase in the Cs^+ K_d values was recorded and above pH 4, a further increase was observed. The maximum K_d for Cs^+ was observed at pH 8.9. pKa values from ~4.2 to 9.0 for illite in a solution with 10% of peat humic acid and a background electrolyte concentration of 0.01 M (Ca^{2+}) have been reported (Martinez et al. 2010). Similarly, for kaolinite, pKa values from 4.4 to 8.8 in the presence of humic acid were reported (Martinez et al. 2010). The conditions in the present study were very similar to those reported for the study of Martinez et al. (2010). In the present study, the proportion of organic matter in the clay layer was 15%, from which the actual proportion of humic acids was unknown, and the overall concentration of major cations in the bog water model solution was 3×10^{-4} M. The sorption on FES sites is independent of pH, but the dissociation of OH groups depends on the solution pH. Thus, the increasing degree of negative charge on the surface hydroxyl groups above pH 4 most likely explains the increase in the observed K_d values of Cs^+ as a function of increasing pH. This also explains the less steep increase in the sorption with increasing pH compared to the increase in the organic layers, as these two components, pH independent and pH dependent sorption, control the uptake of Cs+ in this layer. The increased degree of positive charge on the surface groups in the clay layer at a pH below 4 also most likely explains the increase in the observed clay layer K_d values of I^- between pH 4 and pH 2. For SeO_3^{2-} a similar increase in the clay layer was not seen. Presumably, some proportion of the I^- sorption between oxidized iodine species and organic molecules and I^- and SeO_3^{2-} sorption on protonated nitrogen containing groups through electrostatic interactions also occurs.

10. Conclusions

This study demonstrated that I^- and SeO_3^{2-} sorption in fresh surface moss, peat, gyttja and clay obtained from a nutrient-poor boreal bog is strongly connected to the microbial activity found in this bog and that various abiotic and biotic factors (i.e. pH, clay minerals and plant uptake) affect the behaviour of Cs^+ in the bog. The various biotic and abiotic retention processes affecting the migration of I^-, SeO_3^{2-} and Cs^+ are summarised in Figure 25.

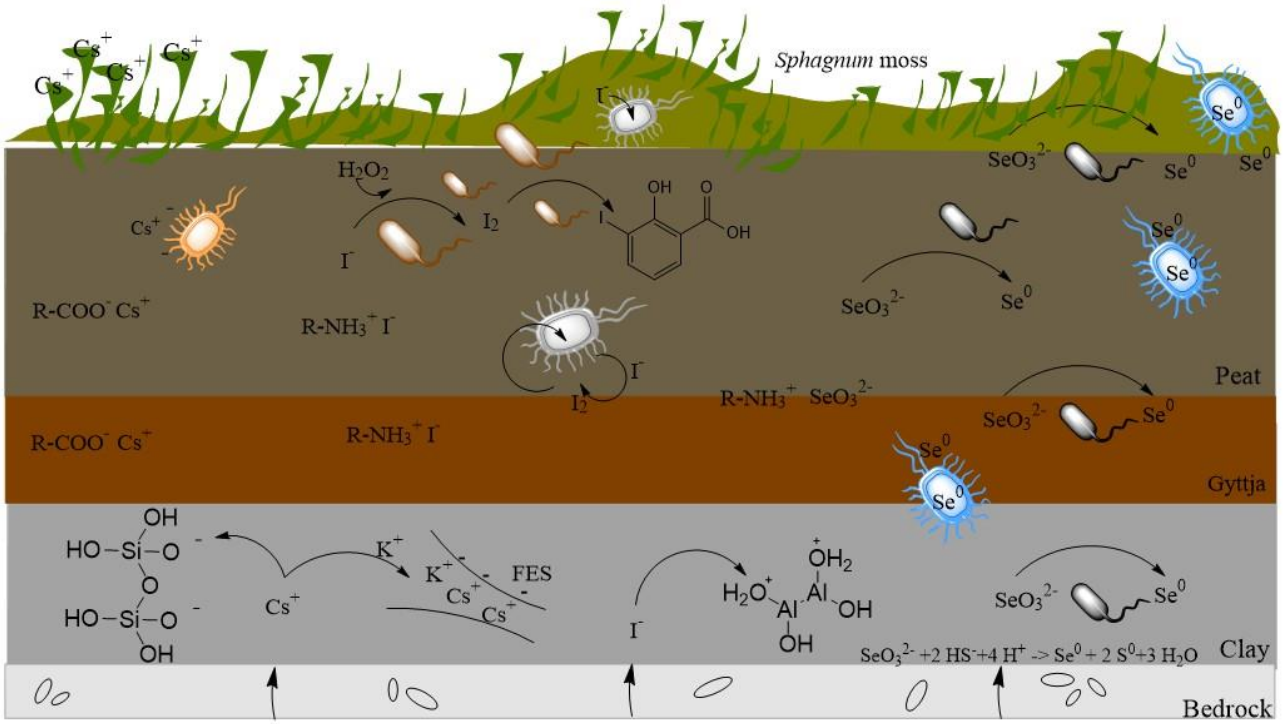


Figure 25. The abiotic and biotic processes occurring in the acidic, nutrient-poor boreal bog affecting the sorption, retention and migration of I^-, SeO_3^{2-} and Cs^+. In the figure the different bog layers are presented in different colours: light grey = bedrock, grey = clay layer, russet = gyttja layer, brown = peat, green = surface moss. The thickness of the layer does not necessarily correspond to the actual situation. The bacteria participating the retention of I^-, SeO_3^{2-} and Cs^+ in the different bog layers are presented in a different way coloured ovals.

As the biosphere safety of the final disposal of SNF is considered, the releases from the bedrock repository will enter the bog from below. At the bottom layer Cs^+ is expected to retain strongly on the clay minerals (illite) found in this layer. The retention of I^- in this anoxic layer is considerably lower and therefore the upward migration of I^- can be expected until an oxic layer in which the microbial oxidation prior to I^- incorporation into the organic matter and oxic accumulation of I^- in bacteria is possible. SeO_3^{2-} on the other hand can be retained in the bottom soil layer through microbial

reduction involving anaerobic bacteria. Limited abiotic reduction with sulfide in the clay layer is expected based on the results obtained for the sterilized samples in which removal of SeO_3^{2-} was decreased in the bottom clay layer. In the organic gyttja and peat layers low level sorption of Cs^+ on the deprotonated carboxylic groups is expected. Similarly, depending on prevailing pH conditions I^- and SeO_3^{2-} can be retained abiotically on protonated nitrogen containing functional groups ($-NH_3^+$) found in organic matter. However the biotic, microbiotically driven retardation of I^- and SeO_3^{2-} in the organic layers is, based on the result obtained for the sterilized samples, expected significantly higher than that induced by the abiotic ion exchange. For SeO_3^{2-} the retardation can include various precipitation mechanisms found in microorganisms (e.g. Painter-type reactions, the thioredoxin reductase system, and sulfide- and siderophoromediated reduction) and reduced Se^0 is expected both inside and outside the cells. I^- on the contrary is expected to be oxidized prior to incorporation both into the organic matter or bacteria. In the oxic surface moss layer, I^- is strongly retained presumably through incorporation into the surface moss after microbial oxidation. Low level accumulation directly into the bacteria cells is also possible. Cs^+ not retained in the bottom clay layer or peat layers may, based to the results obtained for the *in situ* approach be strongly retained in surface moss through plant uptake. In addition low level biosorption on bacteria in all layers can be expected for Cs^+.

The following conclusions can be drawn from this work:

1) *In an acidic nutrient-poor bog, viable microbes are necessary for the sorption of I^-.* This is supported by the observation that the sterilization of samples drastically reduced the sorption of I^- and that the sorption capacity was restored after the incubation of sterilized samples with fresh samples or microbial extract from fresh peat.
2) *Before incorporation into the organic matter, I^- is oxidized into I_2 or HIO in the presence of oxygen.* This is supported by the observation that the sorption of I^- was clearly reduced in anoxic conditions compared to oxic conditions. If no alteration in the speciation of I^- was necessary for the sorption, it should not have been affected by the anoxic conditions. This is because I^- is the most reduced species of iodine.
3) *I^- is converted into I_2 or HIO by microbial oxidation (most likely by bacteria and/or fungi) in presence of oxygen and after oxidation iodine is incorporated into the organic matter.* This is supported by the observation that in the anoxic conditions the I^- K_d values for fresh, untreated samples corresponded to the values obtained for the sterilized samples in oxic conditions. Moreover, both in anoxic conditions and in sterilized samples, the K_d values were significantly lower (<9%) than the values obtained for the fresh, untreated samples. In addition low-level direct bioaccumulation in bacteria is possible.
4) *In an acidic nutrient-poor bog, bacteria are necessary for the removal of SeO_3^{2-} from the solution phase.* This is supported by the observation that sterilization of bog samples inhibited the removal of SeO_3^{2-} from simulated bog water and that bacteria isolated from the bog samples removed SeO_3^{2-} from the solution in the presence of sterilized surface moss, peat, gyttja and clay samples.
5) *SeO_3^{2-} is probably reduced both under oxic and anoxic conditions.* This is supported by the observation that the sorption of SeO_3^{2-} under oxic conditions closely corresponded with the sorption under anoxic conditions.
6) *SeO_3^{2-} is reduced by bacteria present in the bog*. Both abiotic and biotic reduction is possible under anoxic conditions but under oxic conditions bacterial reduction is expected. This is

supported by the observation that SeO_3^{2-} removal from the solution was at the same level both under oxic and anoxic conditions, but was decreased as samples were sterilized and incubated under oxic conditions.

7) *pH and clay minerals affect the sorption of* Cs^+ *in an acidic nutrient-poor bog*. The sorption of Cs^+ was found to increase as a function of increasing pH in all studied layers, and the highest sorption was recorded for the bottom layer of the bog, in which clay minerals (especially illite) were found.
8) *Plant uptake, rhizoidosphere effects and bacteria potentially affect the migration of* Cs^+ *in an acidic nutrient-poor bog.* This is supported by the observation that significantly higher *in situ* K_d values were observed in the surface moss layer compared to the values recorded for the batch experiments, which may be due to the plant uptake and/or rhizoidosphere effects in this layer not being captured by batch experiments. Moreover, bacteria isolated from the bog were able to remove Cs^+ from the solution.

11. Future prospects

In the study presented above new insights into the importance of microbiota for the retention of I^- and SeO_3^{2-} in an acidic, nutrient-poor boreal bog have been brought into question. This can have important applications both in the disposal of spent nuclear fuel as well as in the bioremediation of contaminated sites. Bacteria and fungi can have important functions in the I^- and SeO_3^{2-} retention and migration in the bog environment, typical for boreal, humid regions. The characterization and isolation of fungi from the bog would provide more information of the effect of microorganisms to both the oxidation of I^- and reduction of SeO_3^{2-}. More information of the mechanisms of I^- oxidation and SeO_3^{2-} reduction as well as the proteins involved in their accumulation into the bacterial/fungal biomass is needed in order to construct a more detailed knowledge about I^- and SeO_3^{2-} cycling applicable to a wider environment. In addition, more detailed knowledge about the enzymes participating the I^- and SeO_3^{2-} retention in the bacteria isolated in the present work as well as new isolates of bacteria and fungi would contribute to the understanding of these mechanisms.

In the present work bacteria were shown to also remove Cs^+ from the solution and it was suggested that in the bog environment the plant uptake by *Sphagnum* moss presumably also has an important role in the Cs^+ migration. More detailed knowledge about the uptake of Cs^+ by the fungi and bryophytes in the bog environment is important for the prediction of the migration of Cs^+ in this environment.

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