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# Mineralization of soil colloids by aerobic heterotrophic bacteria of soils

Olga O. Drozdova

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# THÈSE

En vue de l'obtention du

## DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE

Délivré par :

Université Toulouse 3 Paul Sabatier (UT3 Paul Sabatier)

Cotutelle internationale avec  
Université d'Etat de Moscou

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**Présentée et soutenue par :**

**Olga DROZDOVA**

le jeudi 24 décembre 2015

**Titre :**

Minéralisation des colloïdes du sol par les bactéries hétérotrophes aérobies du sol

---

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## **Abstract**

It is known that global climate change has an impact on all components of the hydrobiogeochemical ecological systems. This is especially true for high-latitude boreal ecosystems that are exposed these changes are stronger than others. It is necessary to study these cycles in uncontaminated systems for to understand and quantitatively predict human disturbance of biogeochemical cycles of elements. Among the different regions of the world, the boreal region are one of the largest reservoirs of organic carbon in the peat and soil (organic rich). The release of organic carbon and metals, due to permafrost thawing, and increased runoff of Arctic river (which induced global warming) are likely to change the forms of elements in soil solutions and waters of the rivers and their number that falls falls into the ocean. This is a very important scientific environmental problem.

Multidisciplinary approach for the study of the biogeochemical cycles of the main elements in soils and waters controlled by the activity of microorganisms (by uptake/release of cells or in the process of degradation of dissolved organic matter) was used in the thesis. The innovation of this work is to study the effect of microbial activity (soil typical aerobic bacteria *Pseudomonas*) on the biogeochemical cycles of elements due to take place geochemical, physical, chemical and microbiological processes. The simultaneous study of the biogeochemical cycles of elements and parameters of biological activity will allow us to predict the response of natural systems to climate change and human-induced disturbance (organic or metallic contamination).

Field studies were accompanied by detailed laboratory studies of the reaction mechanisms. The thesis considers to the study of physical and chemical processes using of experimental modeling of the degradation of organic matter in soil extracts and to the study of the quantitative relation between the degradation of organic matter by heterotrophic bacteria *Pseudomonas* and cycles elements.

## Résumé

On sait que les changements globaux climatiques constituent des forçages qui s'exercent sur toutes les composantes hydrobiogéochimiques des systèmes écologiques. Ceci est particulièrement vrai pour les écosystèmes boréaux de haute latitude qui sont des régions très fragiles, fortement soumises au réchauffement climatique. Afin de comprendre et prédire de manière quantitative les perturbations d'origine anthropique des cycles biogéochimiques, il est urgent d'acquérir la connaissance de ces cycles dans les systèmes non pollués «pristines», encore non (ou peu) perturbés. Parmi les différentes régions de monde, les zones boréales représentent un des plus importants réservoirs de carbone organique sous forme de tourbières ou de sols très riches en matière organique. La libération du carbone organique et des métaux en trace associés durant le dégel du permafrost, induite par le réchauffement climatique, constitue le principal changement que subit ce milieu et le principal enjeu scientifique et environnemental. L'augmentation continue du débit des rivières arctiques au cours des dernières décennies, combinée à la libération du carbone et des métaux piégés dans le permafrost, est susceptible de modifier les flux d'éléments vers les océans, mais aussi la spéciation de ces dernières dans les eaux de rivière ou les solutions de sol.

Cette thèse s'inscrit dans une approche résolument pluridisciplinaire, centrée sur l'étude biogéochimique des cycles d'éléments majeurs – éléments en trace dans les eaux contrôlées par l'activité des microorganismes (piégeage/rélargage par la biomasse ou dégradation de la matière organique dissoute et particulaire). Le caractère innovant de cette thèse réside dans l'étude, pour la première fois, de l'impact de l'activité microbienne (minéralisation aérobie par les bactéries typiques des sols, *Pseudomonas*) sur les cycles biogéochimiques des éléments grâce à la mise en œuvre en parallèle d'outils géochimiques, physico-chimiques et microbiologiques. Cette étude simultanée des cycles biogéochimiques des éléments et des paramètres d'activité biologique nous permettra de prédire les réponses des systèmes naturels aux changements climatiques et aux interventions humaines (sous forme de pollution organique ou métallique).



Les études de terrain ont été accompagnés par des études plus détaillées en laboratoire des mécanismes réactionnels. Dans le cadre de ce travail, mes efforts ont été consacrés à l'étude physico-chimique des processus via la modélisation expérimentale de la dégradation de la matière organique des extraits du sol. Et des recherches des relations quantitatives entre la dégradation de la matière organique par les bactéries hétérotrophes *Pseudomonas* et les cycles des éléments associés.

## **Введение**

Известно, что глобальное изменение климата оказывает влияние на все биогеохимические компоненты экологических систем. Это особенно верно для высокоширотных бореальных экосистем, которые сильнее других подвергаются этим изменениям. Чтобы понять и количественно предсказать антропогенные нарушения биогеохимических циклов элементов, необходимо изучать эти циклы в незагрязненных системах, что на данный момент мало исследовано. Среди различных регионов мира, бореальная зона представляет собой один из крупнейших резервуаров органического углерода в торфе и почве, богатой органическими веществами. Высвобождение органического углерода и металлов при таянии вечной мерзлоты и увеличение арктического речного стока в последние десятилетия, связанные с глобальным потеплением, скорее всего, изменит формы нахождения элементов в почвенных растворах и водах рек и их количества, попадающих в океаны. Это является очень важной научной экологической проблемой.

В диссертации развивается междисциплинарный подход, в центре которого находится изучение биогеохимических циклов основных элементов в почвах и водах, контролируемых жизнедеятельностью микроорганизмов (путем захвата/освобождения клетками или в процессе деградации растворенного органического вещества). Научная

новизна данной работы заключается в исследовании влияния микробной активности (типичных почвенных аэробных бактерий *Pseudomonas*) на биогеохимические циклы элементов вследствие происходящих параллельно геохимических, физико-химических и микробиологических процессов. Одновременное изучение биогеохимических циклов элементов и параметров биологической активности позволит нам предсказать ответ природных систем на изменение климата и антропогенное вмешательство (в виде загрязнения органическими веществами и металлами).

Полевые исследования сопровождались более детальными лабораторными исследованиями механизмов реакций. Диссертация, в основном, посвящена изучению физических и химических процессов, с помощью экспериментального моделирования деградации органического вещества в почвенных экстрактах. А также исследованию количественного соотношения между деградацией органического вещества гетеротрофными бактериями *Pseudomonas* и циклами, связанных с ним элементов.

# Chapter 1

## **General introduction**



## 1.1. Background of the study

One of the major goals of the geochemistry is to quantify the element fluxes occurring between different reservoirs (continents and oceans), and to identify the mechanisms which control these fluxes (Ilina, 2012). Boreal regions of the Russian Arctic play a crucial role in transport of elements from continents to the ocean at high latitudes. It is very important to carry out detailed regional studies of elements geochemistry in boreal landscapes.

Soils are highly dynamic systems that are influenced by the interaction between organic and inorganic components and biota. These interactions determine the metal speciation and bioavailability in soil environments (Ledin et al., 1996).

Colloids in surface water originate, in general, from the soil horizons. They can be organic, inorganic and organo-mineral (Viers et al., 1997; Dahlgvist et al., 2007; Pokrovsky et al., 2005, 2006; Andersson et al., 2006; Allard and Derenne, 2007).

The colloids play a major role in the mobilization of trace elements in soils and waters affecting distribution of elements in natural systems (Buffle, 1988). Clays, the oxides, hydroxides and the organic colloids can each have an independent existence in the soil environment, or they can be associated in a very complex colloidal structure (Newman and Hayes, 1990).

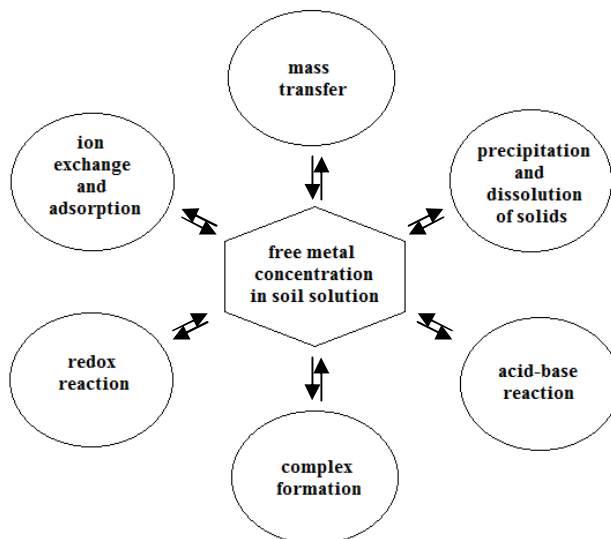
Hydroxides and oxyhydroxides (especially those of aluminium, iron and manganese) and amorphous silicates are important components of the soil inorganic colloidal constituent (Newman and Hayes, 1990). They are formed during intensive chemical weathering of primary soil minerals in the soil. It takes place in an aggressive acid medium created by plant litter decomposition products. This leaching is accompanied by migration and subsequent accumulation of organo-mineral complexes of iron and aluminium in illuvial soil horizon (Lundström et al., 2000).

Degradation of plant litter on the surface of soil yields organic colloids. Humic substances and polisaccharides are the most abundant and important of the organic colloids in soils

(Newman and Hayes, 1990). These materials have a different of acidic functional groups, and especially carboxylic and phenolic groups of varying  $pK_a$  values. The organic colloids are usually combined with mineral soils particles creating organo-mineral clay-humic complexes (i.e., Fe and Al hydroxides stabilized by organic matter).

In soil, metals can be found in several "pools" of the soil, as described by Shuman (1991): (i) dissolved in the soil solution; (ii) occupying exchange sites on inorganic soil constituents; (iii) specifically adsorbed on inorganic soil constituents; (iv) associated with insoluble soil organic matter; (v) precipitated as pure or mixed solids; (vi) present in the structure of secondary minerals and/or primary minerals.

In the soil solution metals exist as either free metal ions, in various soluble complexes with inorganic or organic ligands, or associated with mobile inorganic and organic colloidal material (McKeague et al., 1983). The concentration of metals in the soil solution is governed by a number of interrelated processes, including complexation, oxidation-reduction reactions, precipitation-dissolution reactions and adsorption-desorption reactions (Fig. 1.1).



**Fig. 1.1.** Mechanisms that control concentrations of metal in soils solution (Mattigod, et al., 1981).

Understanding the reactivity of metals in soil is a prerequisite for modeling its transport and fate in soil environment. Adsorption and desorption, precipitation and solubilisation, surface

complex formation, ion exchange, penetration of the crystal structure of minerals, and biological mobilization and immobilization are the major process that control mobility of metals in soil (Chao, 1984).

Quantifying the proportions of metals bound to different soil constituents is important to understand metals behavior and fate in soils. Metals associated with the aqueous phase of soils are subject to movement with soil water, and may be transported through the vadose zone to ground water (McLean and Bledsoe, 1992). Immobilization of metals, by mechanisms of adsorption and precipitation, can prevent movement of the metals to ground water.

The mobility and bioavailability of metals in soil depend on the strength of the bond between metal and soil surface as well as the properties of soil solution (Filgueiras et al., 2002). Changes in soil environmental conditions over time, such as the degradation of the organic waste matrix, changes in pH, redox potential, or soil solution composition, due to various remediation schemes or to natural weathering processes, also may enhance metal mobility (McLean and Bledsoe, 1992).

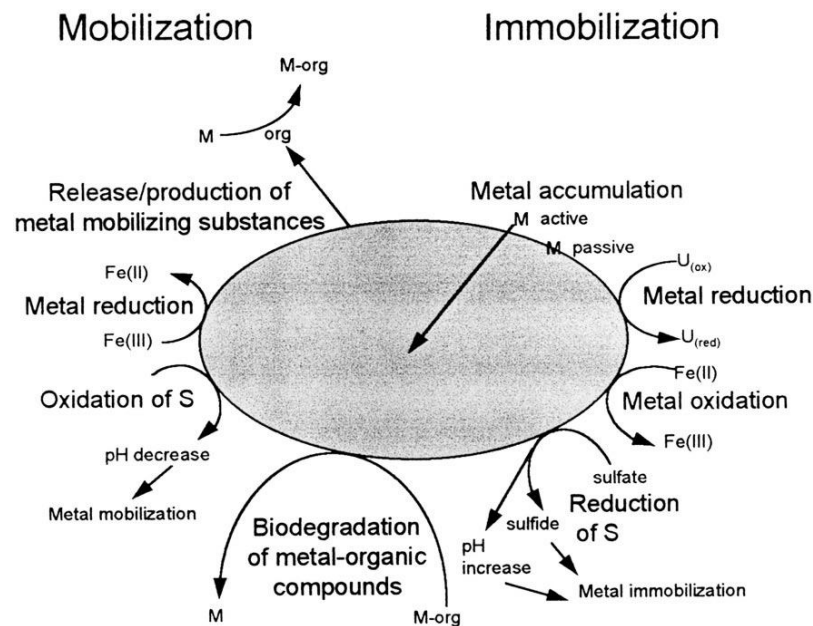
Among the factors controlling the intensities of chemical weathering in soils, solution pH,  $p\text{CO}_2$  and organic compounds produced during enzymatic degradation of vegetation litter or the exometabolism of soil bacteria and roots are believed to be the most important (Barker et al., 1997; Bennett et al., 2001).

Bacteria are abundant in various environments, with common concentrations of  $10^6$  to  $10^9$  cell  $\text{g}^{-1}$  soil (Barns and Nierzwicki-Bauer, 1997). There are several ways in which microorganisms can influence on behaviour of metals in the soils, as described by Ledin (2000) (Fig. 1.2):

- accumulation of metals can occur by metabolism-independent sorption or by intracellular (metabolism-dependent uptake);
- microorganisms can produce or release substances, for example organic compounds that change the mobility of the metals;

- microorganisms participate in the cycling of carbon and influence the amount and character of organic matter. This is important for metal mobility, because organic compounds may bind metals. Microbial degradation of the metal–organic complex can also change the speciation of the metal.

- microorganisms can also influence metal mobility indirectly since they affect pH, Eh, etc.



**Fig. 1.2.** Interactions between metals and microorganisms (Ledin, 2000).

Despite the importance of both the biotic and abiotic soil components in the overall soil affinity for metals, the majority of studies performed so far have been devoted to either the mineral (Bradl, 2004) or bacterial (Fein et al., 1997) components of soil system, without considering the synergetic interaction of metals with both of them.

### *Conclusion*

There are a lot of biogeochemical processes that control metal mobility and bioavailability in the soil.

Bacteria play an important role in the behaviour of metals in the soils. Microbial cell surfaces are providing important reactive interfaces for the adsorption of metals. Biological membranes are sites of uptake, exudation and of a range of enzymatic processes.

Biom mineralization of the soil colloids plays a major role in the mobilization of metals in soils and waters affecting distribution of elements in natural systems.

Ultimately, the understanding of these processes is needed for the accurate and quantitative prediction of the fate and transport of metals on a range of time and length scales.

## **1.2. Study site**

The natural waters of the north of boreal region, North Karelia were chosen as the objects of the study. It is in the Northern Karelia (N 66°, E 30°), ca. 40–60 km south of the Arctic Circle.

The study of pristine river geochemistry, apart of some undeveloped tropical regions and temperate areas of the southern hemisphere, has a tendency to be more and more limited to the subarctic regions (Ilina, 2012). The climate of the region is mild-cold, transitional between oceanic and continental, with a determinant influence of the Arctic and Northern Atlantic air masses. Average temperature is -13°C in January and +15°C in July, but extremes can reach -45° to +35°C in the winter and summer periods, respectively. Average annual precipitation ranges between 450 and 550 mm annually, primarily as rain during the summer months, but also as fog and snow; as evaporation is also low for most of the year, precipitation exceeds evaporation and is sufficient for the dense vegetation growth (Sayre, 1994). The forests of the taiga are largely coniferous, dominated by larch, spruce, fir, and pine, but some broadleaf trees also occur, notably birch, aspen, willow and rowan (Ilina, 2012).

Boreal soils tend to be geologically young and poorly developed and mostly presented by podzols in the European zone. They tend to be acidic due to the decomposition of organic materials (e.g., plant litter) and either nutrient-poor or have nutrients unavailable because of low temperatures (Sayre, 1994). Podzols occur mainly in cool humid climates (McKeague et al., 1983) under forest or heath vegetation in medium textured to coarse material (Steila and Pond, 1989), where conditions favour the development of an organic surface (mor) layer (Lundström et al., 2000).



### 1.3. Objects of the study

The eluvial-humic (surface, organic-rich) and illuvial (mineral, organic-poor) horizons of the podzol soil were used in this study (Fig. 1.3). The samples were collected in July, 2011 in Northern Karelia, within the European boreal zone (N 66°18', E 30°42').



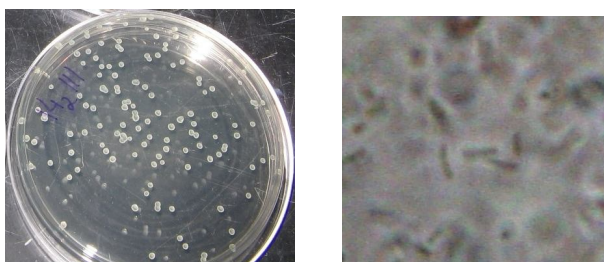
**Fig. 1.3.** Podzol soil (Northern Karelia).

The accumulation of iron and aluminum occurs in illuvial horizon. Such distribution of Fe and Al in the soil profile is typical for this type of soil, due to the different mobility of organic-mineral complexes and colloids. Ni, Mo, Mg, V, Cr, Mn, La, Co and Ti are similar distributions in the profile of podzol. Concentration of these elements connected with illuviation process of iron and manganese compounds. Cu, Zn, Pb and Cd accumulated in the litter of podzol. Firstly, these elements are characterized by low mobility in podzol, and, secondly, the processes of biological accumulation are cause to accumulation of these elements in the surface horizons. The contents of K, Na, Ca, Ba, Sr, and Rb in the soil are higher in the mineral horizons relative to the litter. The total content of carbon of the mineral part of the profile is distributed according to eluvial-illuvial type.

The values of pH of the aqueous extracts increase from 4.9 in the upper horizons to 6.0 - 6.2 in the lower horizons of podzol. The content of inorganic anions ( $\text{Cl}^-$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$  and  $\text{SO}_4^{2-}$ ) is higher in the organic horizons. Dissolved organic carbon decreases from  $69 \text{ mg L}^{-1}$  in the litter to  $12.8 \text{ mg L}^{-1}$  in the lower horizon BC.

The composition of compounds of the elements in the aqueous extracts depends on the features of horizons, the type of soil and vegetation. Migration of elements in podzol depends

significantly on the presence of geochemical barriers. Distribution of water-soluble forms of elements in soil profile is more uniformity relative to the distribution of the total content. Litter is the most probable source the compounds of the elements in surface water.



**Fig. 1.4.** *Pseudomonas aureofaciens* (colony and cells).

The bacterial strain *Pseudomonas aureofaciens* CNMN PsB-03 (Fig. 1.4) was obtained from the Laboratory of Plant Mineral Nutrition and Hydric Regime (Institute of Genetics and Plant Physiology, Moldovan Academy of Sciences, Chisinau, Moldova). It was isolated from chernozem soil and selected due to its capacity to produce exopolysaccharides (EPS) on a sucrose-containing medium.

The basin of the Vostochniy stream was chosen for study. The stream flows from west to east and empties into Lake Tsipringa. The lake is ca. 1 km long with a catchment area is 0.95 km<sup>2</sup> and is at a relative altitude of 50 m. The bedrock of the catchment comprises amphibolitic gabbroids of the low Proterozoic intruzive (Ilina et al., 2013).

#### **1.4. Thesis organisation**

The manuscript is composed of the main thesis based on publications addressing the above mentioned objectives, and the supplementary information.

#### **Chapter 2: Decrease in zinc adsorption onto soil in the presence of EPS-rich and EPS-poor**

*Pseudomonas aureofaciens*. This manuscript is aimed at assessing the sorption of zinc by different soils horizon and influence of bacteria *Pseudomonas aureofaciens* on this process.

**Chapter 3: Impact of heterotrophic bacterium *Pseudomonas aureofaciens* on the release of major and trace elements from podzol soil into aqueous solution.** This manuscript describes the results of study the release of various elements from different horizons of podzol soil in the presence of heterotrophic bacterium *Pseudomonas aureofaciens*.

**Chapter 4: Size fractionation and optical properties of dissolved organic matter in the continuum soil solution-bog-river and terminal lake of a boreal watershed (North Karelia, Russia).** This manuscript is aimed at assessing the change of size fractionation of dissolved organic carbon and its chemical and optical properties in series of filtrates, along the landscape transect from the feeding soil solution and humic bog lake to the stream and to the terminal lake.

**Chapter 5.** There are **conclusions** and **perspectives** of further research.

## References

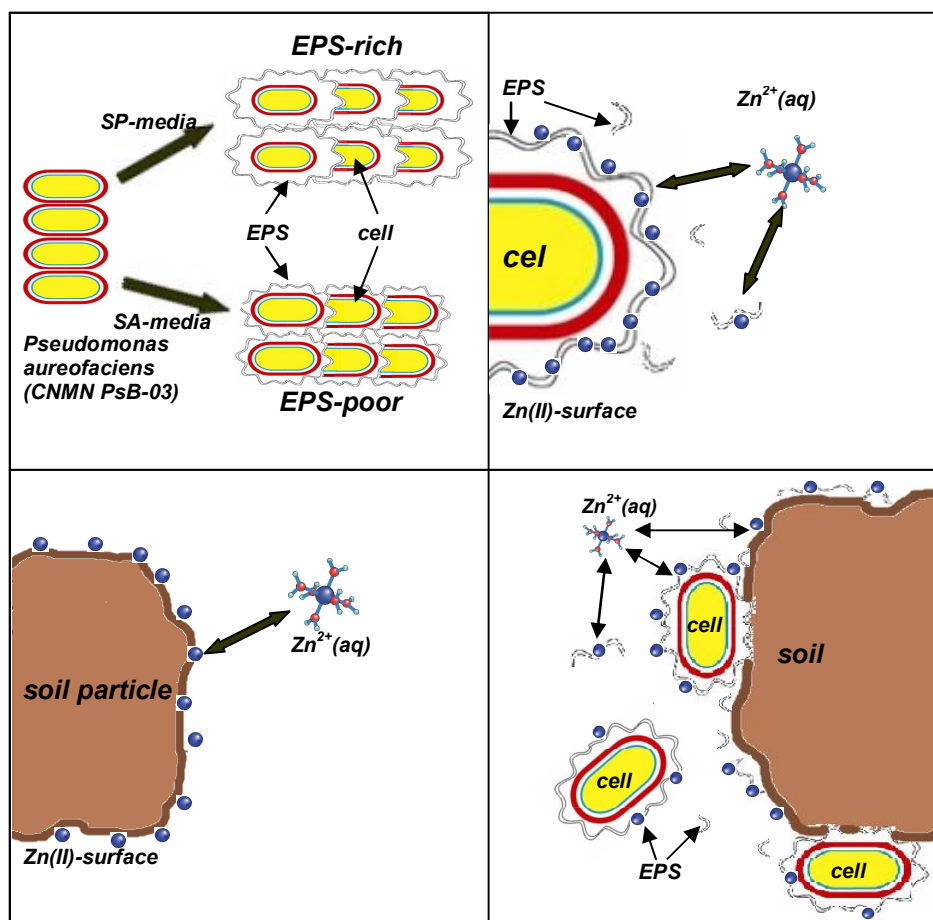
- Allard, B., and Derenne, S. (2007). Oxidation of humic acids from an agricultural soil and a lignite deposit: Analysis of lipophilic and hydrophilic products. *Organic Geochemistry* 38(12), 2036-2057.
- Andersson, K., Dahlgvist, R., Turner, D., Stolpe, B., Larsson, T., Ingri, J., and Andersson, P. (2006). Colloidal rare earth elements in a boreal river: Changing sources and distributions during the spring flood. *Geochimica et Cosmochimica Acta* 70(13), 3261-3274.
- Barns S.M., Nierzwicki-Bauer S.A (1997) Microbial diversity in ocean, surface, and subsurface environments. Geomicrobiology: Interactions Between Microbes and Minerals, in: Banfield, J.F., Nealson, K.H. (Eds.), Reviews in Mineralogy 35 Mineralogical Society of America, Washington, DC, pp. 35–79.
- Barker WW, Welch SA, Banfield JF (1997) Biogeochemical weathering of silicate minerals. In Banfield JF, Nealson KH (ed) Geomicrobiology: Interactions Between Microbes and Minerals. Mineralogical Society of America, Reviews in Mineralogy 35, pp. 391–428.
- Bennett P.C., Rogers J.A., Hiebert F.K., Choi W.J. (2001) Silicates, silicate weathering, and microbial ecology // *Geomicrobiol J.* 18, pp. 3-19.
- Bradl H.B. (2004) Adsorption of heavy metal ions on soils and soils constituents // *Journal of Colloid and Interface Science*, 277, pp. 1–18.
- Buffle, J. (1988). Complexation reactions in aquatic systems: an analytical approach. Ellis Horwood Ltd., Chichester, UK.
- Chao T.T. (1984) Use of partial dissolution techniques in geochemical exploration // *J. Geochem. Explor.*, 20, pp.101-135.
- Dahlgvist, R., Andersson, K., Ingri, J., Larsson, T., Stolpe, B., and Turner, D. (2007). Temporal variations of colloidal carrier phases and associated trace elements in a boreal river. *Geochimica et Cosmochimica Acta* 71(22), 5339-5354.
- Fein J.B., Daughney C.J., Yee N., Davis T.A. (1997) A chemical equilibrium model for metal adsorption onto bacterial surfaces // *Geochim. Cosmochim. Acta*, 61, pp. 3319-3328.
- Filgueiras A.V., Lavilla I., Bendicho C. (2002) Chemical sequential extraction for metal portioning in environmental solid samples // *J. Environ. Monit.* 4, pp. 823-857.
- Ilina S.M. (2012) Rôle de la matière colloïdale dans le transfert des éléments chimiques en milieu boréal. Thesis PhD, Hydrologie, hydrochimie, sol et environnement, Université Paul Sabatier – Toulouse III, 313 p.
- Ilina S.M., Viers J., Lapitsky S.A., Mialle S., Mavromatis V., Chmeleff J., Brunet P., Alekhin Yu.V., Isnard H., Pokrovsky O.S. (2013). Stable (Cu, Mg) and radiogenic (Sr, Nd) isotope fractionation in colloids of boreal organic-rich waters. *Chemical Geology* 342, 63–75.

- Ledin M., Krantz-Rolcker C., Allard B. (1996) Zn, Cd and Hg accumulation by microorganisms, organic and inorganic soil components in multi-compartment systems // *Soil Biol. Biochem.* 28 (6), pp. 791–799.
- Ledin M. (2000) Accumulation of metals by microorganisms — processes and importance for soil systems // *Earth-Science Reviews* 51, pp. 1–31.
- Lundström U.S., van Breemen N., Bain D. (2000) The podzolization process. A review // *Geoderma* 94, pp. 91–107.
- Mattigod S. V., Sposito G., Page A. L. (1981) Factors affecting the solubilities of trace metals in soils. In D. E. Baker (Ed.). *Chemistry in the soil environment*. ASA Special Publication No 40. Amer. Soc. Agronomy, Madison, WI.
- McKeague J.A., De Connick F., Franzmeier D.P. (1983) Spodosols. In: Wilding, L.P., Smeck, N.E., Hall, G.F. (Eds). *Pedogenesis and Soil Taxonomy*. Elsevier, New York, pp. 217–252.
- McLean J.I., Bledsoe B.E. (1992) Behavior of Metals in Soils // *Ground Water Issue*, EPA/540/S-92/018.
- Newman A.C.D., Hayes M.H.B. (1990) Some properties of clays and of other soil colloids and their influences on soils, in De Boodt M.F., Hayes M.H.B., Herbillon A., De Strooper E.B.A., Tuck J.J. (Eds), *Soil colloids and their associations in aggregates*, New York, pp. 39-57.
- Pokrovsky, O.S., Dupré, B., and Schott, J. (2005). Fe–Al–organic Colloids Control of Trace Elements in Peat Soil Solutions: Results of Ultrafiltration and Dialysis // *Aquatic Geochemistry* 11(3), 241-278.
- Pokrovsky, O.S., Schott, J., and Dupre, B. (2006). Trace element fractionation and transport in boreal rivers and soil porewaters of permafrost-dominated basaltic terrain in Central Siberia // *Geochim. Cosmochim. Acta*, 70, 3239–3260.
- Sayre A. (1994). *Taiga*, New York: Twenty-First Century Books.
- Steila D., Pond T.E. (1989) *The Geography of Soils, Formation, Distribution, and Management*. 2nd edn. Rowman and Littlefield, Savage, NJ.
- Stevenson F.J., Ardakani M.S. (1972) A modified ionexchange technique for the determination of stability constants of metal-soil organic matter complexes // *Soil Sci. Soc. Am. Proc.* 36: 884-890.
- Shuman L. M. (1991) Chemical forms of micronutrients in soils. In J. J. Mortvedt (ed.). *Micronutrients in agriculture*. Soil Soc. Soc. Amer. Book Series #4. Soil Sci. Soc. Amer., Inc., Madison, WI.
- Viers, J., Dupre, B., Polve, M., Dandurand, J., and Braun, J. (1997). Chemical weathering in the drainage basin of a tropical watershed (Nsimi-Zoetele site, Cameroon): comparison between organic-poor and organic-rich waters // *Chem. Geol.* 140, 181-206.

# Chapter 2

## Decrease in zinc adsorption onto soil in the presence of EPS-rich and EPS-poor *Pseudomonas aureofaciens*

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The goal of this study is to estimate of bacteria–metal–soil mechanisms of reactions in natural systems. In this paper we investigated process adsorption of zinc onto the soil in the presence of gram negative bacteria.

There are a lot of studies that have investigated the adsorption of metal on soil or bacteria systems. However, the adsorption behavior of a metal in a system containing both bacteria and soil may be different from that predicted based on stability constants for the binary bacteria–metal and soil–metal complexation reactions. Microbial cell surfaces are providing important reactive interfaces for the adsorption of metals. Bacteria can affect the speciation of metal cations in soil through different reactions. These reactions can alter the mobilities and biogeochemical behavior of both the metal and organic ligands involved.

In this study, we conducted adsorption experiments in ternary system involving bacteria, zinc cations and soil. We performed a variety of batch adsorption experiments in binary (bacteria–zinc, soil–zinc) and ternary (bacteria –zinc–soil) systems.

Two contrasting soil horizons of the podzol soil were used in this research study. The differences between two soil horizons are linked to differences in the content of the mineral and organic components.

# Decrease in zinc adsorption onto soil in the presence of EPS-rich and EPS-poor *Pseudomonas aureofaciens*

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## Abstract

The adsorption of Zn onto the humic and illuvial horizons of the podzol soil in the presence of soil bacteria was studied using a batch-reactor technique as a function of the pH (from 2 to 9) and the Zn concentration in solution (from 0.076 mM to 0.760 mM). Exopolysaccharides-forming aerobic heterotrophs *Pseudomonas aureofaciens* were added at 0.1 and 1.0 g<sub>wet</sub> L<sup>-1</sup> concentrations to two different soil horizons, and Zn adsorption was monitored as a function of the pH and the dissolved-Zn concentration. The pH-dependent adsorption edge demonstrated more efficient Zn adsorption by the humic horizon than the mineral horizon at otherwise similar soil concentrations. The Zn adsorption onto the EPS-poor strain was on slightly lower than that onto EPS-rich bacteria. Similar differences in the adsorption capacities between the soil and bacteria were also detected by “langmuirian” constant-pH experiments conducted in soil-Zn and bacteria-Zn binary systems. The addition of 0.1 g<sub>wet</sub> L<sup>-1</sup> *P. aureofaciens* to a soil-bacteria system (4 g<sub>dry</sub> L<sup>-1</sup> soil) resulted in statistically significant decrease in the adsorption yield, which was detectable from both the pH-dependent adsorption edge and the constant-pH isotherm experiments. Increasing the amount of added bacteria to 1 g<sub>wet</sub> L<sup>-1</sup> further



decreased the overall adsorption in the full range of the pH. This decrease was maximal for the EPS-rich bacteria and minimal for the EPS-poor bacteria (a factor of 2.8 and 2.2 at pH = 6.9, respectively). These observations in binary and ternary systems were further rationalized by linear-programming modeling of surface equilibria that revealed the systematic differences in the number of binding sites and the surface-adsorption constant of zinc onto the two soil horizons with and without bacteria. The main finding of this work is that the adsorption of Zn onto the humic soil-bacteria system is lower than that in pure, bacteria-free soil systems. This difference is statistically significant ( $p < 0.05$ ). As such, EPS-rich bacteria are capable of efficiently shielding the soil particles from heavy-metal adsorption. The removal efficiency of heavy metals in an abiotic organic-rich soil system should therefore be significantly higher than that in the presence of bacteria. This effect can be explained by the shielding of strongly bound metal sites on the organic-rich soil particles by inert bacterial exopolysaccharides.

**Keywords:** *humic, podzol, zinc, surface, EPS, bacteria, protection, modeling*

## **2.1. Introduction**

Soils are highly dynamic systems that are influenced by the interaction between organic and inorganic components and biota. These interactions determine the metal speciation and bioavailability in soil environments [1]. Despite the importance of both the biotic and abiotic soil components in the overall soil affinity for heavy metals, the majority of studies performed so far have been devoted to either the mineral [2] or bacterial [3] components of soil system, without considering the synergetic interaction of metals with both of them. Among various divalent metals, zinc is particularly interesting because it combines the properties of a contaminant/toxicant of high environmental concern and an essential micronutrient for soil microorganisms and plants. It is known that the soil capacity of Zn adsorption depends on a number of physicochemical properties, notably the pH of the soil solution [4–9]. The pH of the

interstitial soil solution controls the degree of ionization, the surface charge, the adsorption mechanisms and the capacity for metal adsorption. Among the main factors controlling the Zn adsorption onto soil are the mineral components [5], the organic matter [10], the content of carbonate minerals [11] and the total zinc loading [12]. Soil organic matter (SOM) strongly controls the physical, chemical and biological conditions of the soils and provides a significant buffer capacity [13]. For example, from 20% to 70% of the exchange capacity of the soil is due to humic substances [14].

Bacteria are abundant in various environments, with common concentrations of  $10^6$  to  $10^9$  cell  $g^{-1}$  soil [15]. The high surface area of soil microorganisms allows them to interact easily with dissolved metals; as a result, bacterial surfaces are capable of greatly controlling the overall metal mobility in aqueous and soil systems [16, 17]. Numerous studies have evaluated the role of microbial surface-adsorption processes compared to other microbial processes on the overall accumulation of metals in soil [18–20]. Over past decades, cation adsorption onto bacterial surfaces has been extensively studied using laboratory and field approaches [3, 21–23]. The most common functional groups on the bacterial surface responsible for metal binding are carboxylate, hydroxyl and phosphoryl groups [24, 25].

Many bacteria are capable of synthesizing external exopolysaccharides (EPS). Molecules of EPS from most bacteria are negatively charged due to the presence of carboxylate and phosphorylate moieties [26]. Although EPS are known to efficiently take up metal ions from aqueous solutions [27], the protective role of EPS on metal adsorption onto cell envelopes has also been demonstrated ([28] and references therein). In contrast to a significant number of studies devoted to metal adsorption onto various soil fractions, cell surfaces and bacterial exopolysaccharides [29–33], works on adsorption in ternary systems of soil-microorganisms-metals are quite rare [cf., 34]. The necessity for such studies stems from the double role of the majority of bacterial EPS: on the one hand, EPS are efficient metal scavengers, and on the other, they protect cell membranes and interior compartments from aggressive external milieu via

decreasing the amount of metal adsorbed onto EPS-rich cells compared to EPS-poor ones. Moreover, abundant and rather inert EPS may shield not only the native bacterial cells in soil environments but also decrease the availability of soil mineral and organic particles for metal adsorption. As a result, bacteria-bearing soil may be a less efficient metal adsorbent than abiotic soil systems. The main goal of this study was to test this hypothesis for two contrasting soil horizons with a typical aerobic-soil, heterotrophic bacterium, *Pseudomonas aureofaciens*, producing abundant EPS. To this end, we experimentally studied Zn surface adsorption in individual (soil + Zn, bacteria + Zn) and complex (soil + bacteria + Zn) systems. Using thermodynamic modeling of surface equilibria, we demonstrate a measurable and rather unexpected decrease in the Zn adsorption for the soil + bacteria system relative to the binary system. It follows that abiotically treated soils represent the most efficient natural sorbent for Zn under a wide range of environmental conditions.

## **2.2. Materials and methods**

### *2.2.1. Soil samples*

The eluvial-humic (surface, organic-rich) and illuvial (mineral, organic-poor) horizons of the podzol soil were used in this research study. The samples were collected in July, 2011 in Northern Karelia, within the European boreal zone (N 66°18', E 30°42'). After sampling, the roots were manually removed. The soils were air-dried and autoclaved at 121 °C for 2 h. Table 2.1 gives selected chemical properties of the soil samples. Conventional chemical analyses of the soils were performed as described elsewhere [35]. The specific surface areas were equal to 0.08 and 1.04 m<sup>2</sup> g<sup>-1</sup> for the humic and illuvial soil horizons, respectively, as determined by multi-point Kr adsorption using the BET method. This result can be understood given higher proportion of clays and Fe oxide in the mineral horizon. In contrast, dry amorphous humic material may not be suitable for Kr adsorption: the ultra-microporous structure of soil organic matter may block the passage of Kr molecules inside the bulk of the material.

**Table 2.1.** Selected properties of the soil samples.

Soil horizon	pH <sup>a</sup>	CEC <sup>b</sup> , mmol kg <sup>-1</sup>	% OC <sup>c</sup>	% Fe <sub>2</sub> O <sub>3</sub>	% Al <sub>2</sub> O <sub>3</sub>
humic	5.6	9.1	2.1	1.2	7.6
illuvial	6.0	1.6	0.6	2.2	11.1

<sup>a</sup> Measured at a soil-to-solution ratio of 1:2.5

<sup>b</sup> The cation-exchange capacity

<sup>c</sup> Organic carbon

### 2.2.2. Culture characterization

The bacterial strain *P. aureofaciens* CNMN PsB-03 was obtained from the Laboratory of Plant Mineral Nutrition and Hydric Regime (Institute of Genetics and Plant Physiology, Moldovan Academy of Sciences, Chisinau, Moldova). It was isolated from chernozem soil and selected due to its capacity to produce exopolysaccharides (EPS) on a sucrose-containing medium [36]. The strain of *P. aureofaciens* was maintained at 4 °C in liquid succinic acid (SA) media and cultured in two different media: (1) a sucrose/peptone (SP) solution, thus promoting rich EPS synthesis; or (2) an SA media, yielding very poor EPS production [28]. The cultivation was performed at 25 °C for 48–72 h with continuous shaking until the beginning of the stationary growth phase. The experiments were conducted using rinsed bacterial biomass, which was produced by repeated centrifugation (three times) in an inert electrolyte solution of 0.01 M NaCl.

Statistical treatment of Zn and DOC concentrations included the use of best fit functions based on the method of least squares and Pearson correlation. In this method,  $P < 0.05$  means the difference between concentration values is important and statistically significant. On the other hand,  $P > 0.05$  means that the differences in the values are not statistically significant and they may stem from random samples variability.

### 2.2.3 Metal adsorption

Two types of experiments were performed: (i) adsorption of zinc as a function of the pH and (ii) adsorption of zinc at a constant pH as a function of the zinc concentration in the solution. All of the experiments were performed in the solution undersaturated conditions with respect to

any metal oxide or hydroxide, as verified by calculations with the MINTEQA2 computer code and corresponding database [37].

The initial zinc concentration was fixed at 0.06 mM for the variable-pH experiments and the zinc concentration was varied between 0.076 mM and 0.760 mM for the constant-pH experiments. The pH was adjusted by adding aliquots of 0.1 M NaOH or 0.1 M HCl. The pH was also maintained via a NaHEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt) buffer, which was added to a final concentration of 0.02 M.

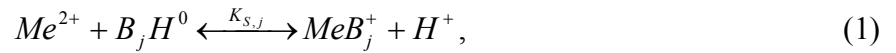
The adsorption experiments were conducted over 24 h at  $25 \pm 2$  °C in a continuously agitated suspension with an ionic strength of 0.01 M NaCl, using 30-mL sterile polypropylene vials. Each experiment was run in triplicate. The soil concentrations in the experiments were maintained at  $4 \text{ g L}^{-1}$ , and the biomass values of the bacteria were 0.1 and  $1 \text{ g L}^{-1}$ . At the end of each experiment, the suspension was centrifuged at 4500g, and the resulting supernatant was filtered through a 0.45- $\mu\text{m}$  acetate cellulose filter, acidified with ultrapure  $\text{HNO}_3$  and stored in the refrigerator until the analysis. The adsorption of zinc onto soils and cell walls for both EPS-rich and EPS-poor *P. aureofaciens* was investigated in the pH range between 2 and 9. The adsorption of zinc was quantified by subtracting, at each solution pH, the concentration of zinc remaining in suspension from concentration of added zinc in the supernatant (control experiments were performed without soils and bacteria). The adsorption of zinc onto the vial walls in the full range of the studied pH was negligible (typically between 2% and 5% of added Zn concentration but never higher than 8%) as verified by various blank experiments. This blank adsorption was explicitly taken into account then calculating the adsorption yield.

All filtered solutions were analyzed for their aqueous Zn concentrations using flame atomic absorption spectroscopy (Perkin Elmer AAnalyst 400) with an uncertainty of  $\pm 2\%$  and a detection limit of  $0.05 \text{ mg L}^{-1}$ . The dissolved organic carbon (DOC) concentration in solution was monitored in all experiments of the pHdependent adsorption edge, at a pH value from 2 to

11, using a Carbon Total Analyzer (Shimadzu TOC-6000) with an uncertainty of 3% and a detection limit of 0.05 mg L<sup>-1</sup>.

#### 2.2.4. Linear Programming Model (LPM)

The non-electrostatic linear-programming model (LPM) was applied for pH-dependent adsorption edge and constant-pH adsorption edge experiments to compute the apparent equilibrium constants and the site densities for each experiment [28, 38, 39]. This model is convenient for describing complex, multicomponent and multilayer systems [40–42]. The interaction of metal cations (Me<sup>2+</sup>) and protons with the bacterial surface in the pH-dependent adsorption edge experiments is represented by:



where  $B_j$  represents a surface-reactive site, and  $K_{S,j}$  stands for the concentration apparent equilibrium constant conditional on the ionic strength. For a  $j$ -th deprotonated binding site at the  $i$ -th pH value,  $K_{S,j}$  can be defined as:

$$K_{S,j} = \frac{[MeB_j^+]_i \cdot [H^+]_{meas,i}}{[Me^{2+}]_{meas,i} \cdot [B_j H^0]_i}, \quad (2)$$

where  $i = 1 \dots n$  titrant additions and  $j = 1 \dots m$  binding sites. In the above expression,  $K_{S,j}$  is a function of the experimentally determined proton and metal concentrations, ( $[H^+]_{meas,i}$  and  $[Me^{2+}]_{meas,i}$ ) and of the amount of Me<sup>2+</sup> bound to the  $j$ -th site at the  $i$ -th pH value,  $[MeB_j^+]_i$ . For pH-dependent Me adsorption edge experiments, an average of two metal binding groups were usually found by the LPM approach (i.e., two site-density values  $[B_j]$ , assigned to  $pK_s$  values on the LPM fix grid).

For constant-pH adsorption isotherm data, the LPM model assumes the following equation:



where  $B_j$  represents a specific surface functional group, and  $K_{m,j}$  the apparent metal-ligand binding constant conditional on the ionic strength. For a  $j$ -th deprotonated functional group at a fixed pH value,  $K_{m,j}$  can be defined as:

$$K_{m,j} = \frac{[MeB_j^+]_i}{[Me^{2+}]_{meas,i} \cdot [B_j]_i}, \quad (4)$$

where  $i = 1 \dots n$  ligand additions and  $j = 1 \dots m$  binding sites. In the above expression,  $K_{m,j}$  is a function of the experimentally determined metal concentrations, ( $[Me^{2+}]_{meas,i}$ ), and of the amount of  $Me^{2+}$  bound to the  $j$ -th site as a function of increasing biomass and at a fixed pH value,  $[MeB_j^+]_i$ . Note that the values of  $K_{s,j}$  and  $K_{m,j}$  found in this study for pH-edge and fixed-pH metal complexation experiments are not directly comparable because  $K_S$  is a function of  $K_m$ :

$$K_S = K_m \cdot K_a, \quad (5)$$

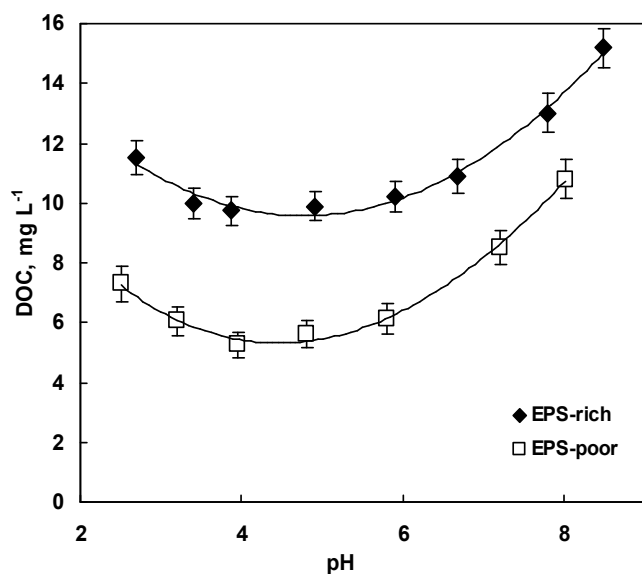
where  $K_a$  is the acidity constant of a specific functional group on the bacterial surface [28, 38]. However, because the acid/base properties of the studied soils are not known from surface-titration experiments, the relationship between  $K_S$  and  $K_m$  cannot be quantified.

## 2.3. Results and Discussion

### 2.3.1. DOC concentration in adsorption experiments

The dissolved organic carbon concentration in the experiments of the Zn adsorption onto soils and bacteria is illustrated as a function of the pH in Figs. 2.1–2.3. The DOC concentration in the bacterial experiments with  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$  *P. aureofaciens* ranged from 9.7 to 15.2 and from 5.2 to 10.8  $\text{mg L}^{-1}$  for the EPS-rich and EPS-poor strains, respectively. A DOC concentration that was factor of 1.5 higher in experiments with the EPS-rich cultures compared to the EPS-poor cultures may be linked to the degradation and release of surface EPS layers, which is known to be the main source of DOC in experiments with bacterial cells [43]. The increase in the [DOC] with the pH increase at  $\text{pH} > 6$  (Fig. 2.1) may be linked with the desorption of the notably hydrophobic fractions [44, 45], whereas the [DOC] increase with the pH decrease at  $\text{pH} < 4$

suggests the degradation of both the cell surface EPS and partial leaching of the underlying organic layers.

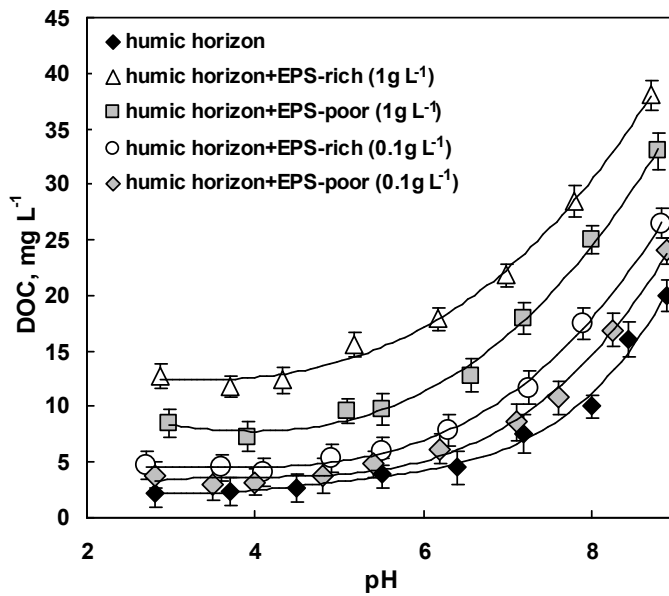


**Fig. 2.1.** Dissolved organic carbon during experiments of zinc adsorption on surface of *Pseudomonas aureofaciens* as a function of pH. The biomass of bacteria is 1 g<sub>wet</sub> L<sup>-1</sup>.

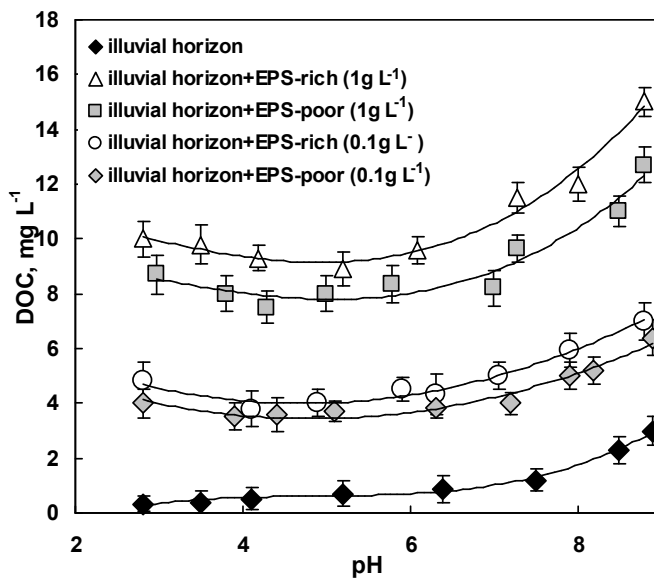
In experiments with soil horizons, the [DOC] ranged from 2.2 to 20.0 and from 0.3 to 3.0 mg L<sup>-1</sup> for the humic (organic) and illuvial (mineral) horizons, respectively, as illustrated in Figs. 2.2 and 2.3. A systematic increase in the DOC with the pH rise is mostly due to the increase in the solubility of the humic soil fulvic fractions in alkaline solutions [14]. This increase is significantly higher in the organic-rich soil horizon, which exhibits a factor of 3 higher SOM content than the illuvial horizon (Table 2.1). It is noteworthy that, despite the significantly lower SSA<sub>BET</sub> of the organic horizon, the DOC release appears to be insensitive to the total surface area available in the reaction because the latter is determined essentially by the soil clay minerals.

Finally, in experiments with both soils and bacteria, the DOC concentration is rather similar to those in pure bacterial experiments and significantly higher than the [DOC] in bacteria-free soil experiments. With the increase of the *P. aureofaciens* biomass from 0.1 to 1.0 g<sub>wet</sub> L<sup>-1</sup>, the DOC systematically increases, by a factor of 2.5 and 3.0 for the mineral and humic horizons, respectively. This increase is 20% to 30% higher (statistically significant at  $p < 0.05$ ) for the EPS-rich strain than for the EPS-poor strain (Fig. 2.3).





**Fig. 2.2.** Dissolved organic carbon during experiments of zinc adsorption on humic horizon and surface of *Pseudomonas aureofaciens* as a function of pH. Soil concentration is 4 g<sub>dry</sub> L<sup>-1</sup> in all experiments.

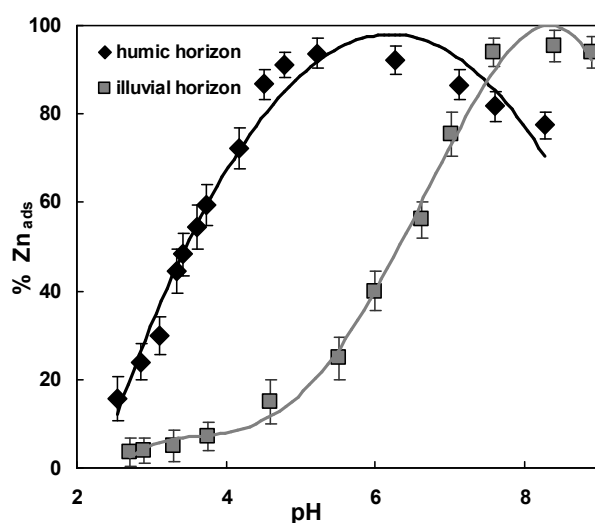


**Fig. 2.3.** Dissolved organic carbon during experiments of zinc adsorption on illuvial horizon and surface of *Pseudomonas aureofaciens* as a function of pH. Soil concentration is 4 g<sub>dry</sub> L<sup>-1</sup> in all experiments.

### 2.3.2. Adsorption of zinc as a function of the pH

The pH-dependent Zn adsorption edges for humic and illuvial soil horizons are shown in Fig. 2.4. The increase in the adsorption % starts at pH values below 3 for organic-rich soil and at pH values above 4 for the mineral soil horizon. The maximal adsorption values of Zn, close to

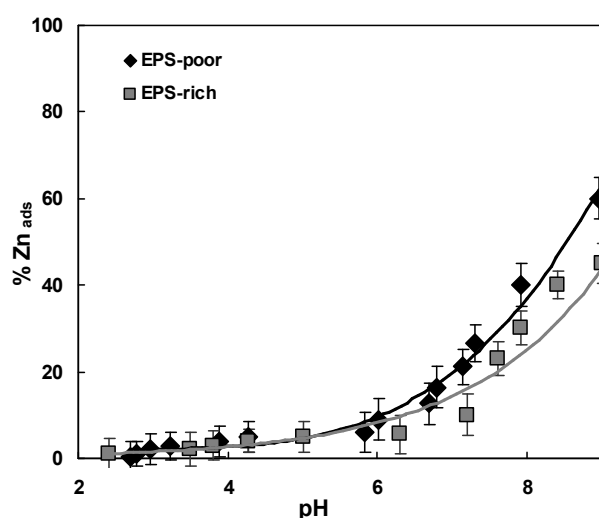
95%, are achieved at pH values of 4.7 and 7.7 for the organic and mineral horizons, respectively. An increase in the pH is known to increase the total number of negative surface charges on both the mineral and the organic components of soils, thus increasing the total adsorption capacity for Zn [46, 47]. The observed difference in the adsorption edges between two soil horizons are presumably linked to differences in the dissociation constants of the functional groups in the humic components and at the mineral surfaces [2, 48]. The slow rise of the Zn adsorption curve on the illuvial horizon may be linked to the competition between  $Zn^{2+}$  and  $H^+$  or  $Al^{3+}$  at  $pH < 5.3$  to 5.5 [49, 50]. Note that, after achieving the maximal adsorption, a further rise in the pH induces remobilization of Zn in solution and a decrease in the adsorption percentage, which is especially pronounced for the humic horizon at pH values greater than 6.5 (Fig. 2.4). This can be explained by Zn complexation with humic acids, as is also confirmed by the [DOC] increase at  $pH > 6$  (Figs. 2 and 3) and can be adequately reproduced by LPM, assuming Zn complexation with dissolved carboxylates (see Section 3.4 below).



**Fig. 2.4.** Percentage of zinc adsorbed on soil as a function of the pH. Soil concentration is  $4 \text{ g}_{\text{dry}} \text{ L}^{-1}$ , and the initial Zn concentration is  $0.06 \text{ mM}$ . Lines represent the LPM model results with the parameters listed in Table 2.3 (see Section 3.4 below).

Adsorption of Zn onto EPS-rich and ESP-poor *P. aureofaciens* cell surfaces as a function of the pH is shown in Fig. 2.5. A very slow rise in the adsorption% with pH at pH values below 6 may be due to cell aggregation linked to surface-group protonation and a decrease in the

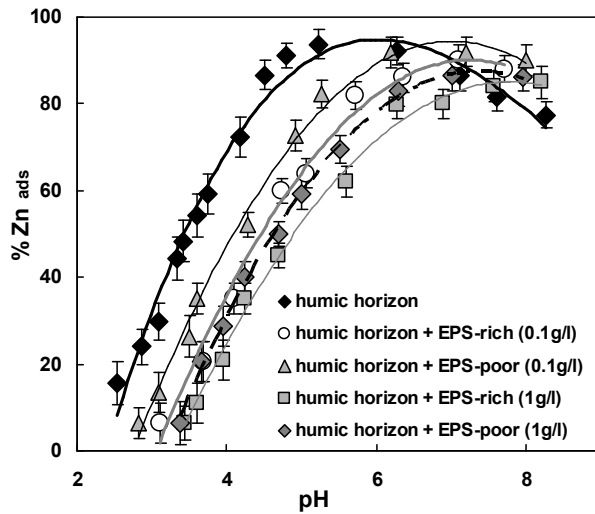
electrostatic repulsion of the cells. The adsorption is slightly lower for the EPS-rich strain than for the EPS-poor one. This difference is statistically significant ( $p < 0.05$ ) only at pH above 8. It is possible that the cell aggregation in a suspension increases with an increase in the EPS concentration; as a result, there is a decrease in the cell surface area available for metal adsorption, as is known for other metals on *P. aureofaciens* [28] and for other bacteria [43]. Another reason for the difference between the EPS-rich and EPS-poor strains is the complexation of  $Zn^{2+}$  with soluble EPS [51]. Because DOC, an indicator of the soluble EPS concentration, in a suspension of the EPS-rich strain is significantly higher than that in the EPS-poor strain (Fig. 2.1), such complexation is capable of decreasing the concentration of free  $Zn^{2+}$  available for adsorption onto cells [43].



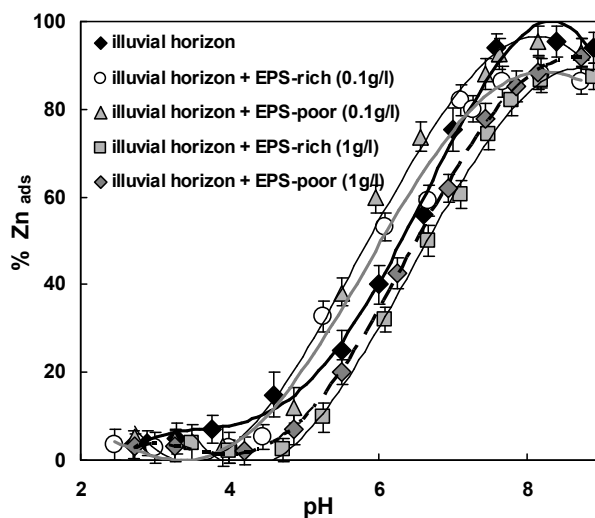
**Fig. 2.5.** Percentage of zinc adsorbed onto the surface of *P. aureofaciens* as a function of the pH. The biomass of the bacteria is  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ , and the initial Zn concentration is  $0.06 \text{ mM}$ . Lines represent the LPM model results.

The results of the Zn adsorption experiments in the presence of two adsorbents are illustrated in Figs. 2.6 and 2.7. The general features of pH dependence remain the same as in the abiotic systems. It can be seen that Zn is preferentially adsorbed by the soil surfaces rather than by bacterial cells. The soil humic horizon experiment demonstrated the maximum of adsorption (90–92%) at a pH value close to 7 at  $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$  of bacteria; this maximum is close to 85–86% at a pH of 7.5–8.0 and  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$  of bacteria. This difference is statistically significant ( $p < 0.05$ ).

For the illuvial horizon, the addition of bacteria does not significantly (at  $p < 0.05$  level) modify the maximal adsorption value.



**Fig. 2.6.** Percentage of zinc adsorbed onto the humic horizon of podzol soil and the surfaces of *P. aureofaciens* as a function of the pH. The soil concentration is equal to  $4 \text{ g}_{\text{dry}} \text{ L}^{-1}$  in all experiments, and the initial Zn concentration is 0.06 mM. Lines represent the LPM model results.



**Fig. 2.7.** Percentage of zinc adsorbed onto the illuvial horizon of podzol soil and the surfaces of *P. aureofaciens* as a function of the pH. The soil concentration is equal to  $4 \text{ g}_{\text{dry}} \text{ L}^{-1}$  in all experiments, and the initial Zn concentration is 0.06 mM. Lines represent the LPM model results with parameters listed in Table 2.3.

The difference between soils and bacteria could be partially explained by Zn complexation in solution by the EPS ligands (DOC) excreted by bacterial cells (cf. Fig. 2.2). The DOC complexes  $\text{Zn}^{2+}_{(\text{aq})}$  and thus decreases free Zn concentration available for adsorption.

However, the sole effect of aqueous EPS cannot explain the decrease of Zn adsorption onto soils in the presence of *P. aureofaciens*. The [DOC] increase in bacteria + soil system relative to soil suspension is much higher for the illuvial horizon compared to the humic horizon (Figs. 2.3 and 2.2, respectively), yet the Zn adsorption decrease is the highest for organic soil horizon (compare Figs. 2.6 and 2.7). These differences are statistically significant at  $p < 0.05$ .

### 2.3.3. Adsorption of zinc as a function of the zinc concentration in solution

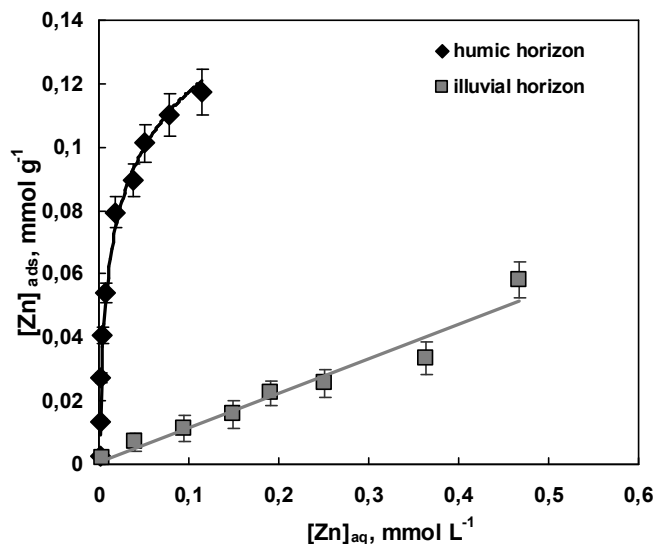
The adsorption of zinc onto soil and the surface of bacteria was studied at a constant pH  $6.9 \pm 0.1$ , in the range of Zn concentrations from 0.076 mM to 0.76 mM. The soil concentration in the experiments was maintained at  $4 \text{ g}_{\text{dry}} \text{ L}^{-1}$ , and the biomass of the bacteria was 0.1 or  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ . The Langmuir isotherm has been shown to be suitable for interpreting Zn adsorption studies [52, 53]. It was used to describe the adsorption data according to Eqn. (6):

$$q = \frac{q_{\max} \cdot K_L \cdot c}{1 + K_L \cdot c}, \quad (6)$$

where  $K_L$  is the Langmuir equilibrium ( $\text{g mmol}^{-1}$ ) constant, and  $q_{\max}$  is the maximum adsorption capacity ( $\text{mmol g}^{-1}$ ). This equation provided an adequate fit to the data with  $R^2 > 0.98$ ; the obtained langmuirian parameters ( $K_L$  and  $q_{\max}$ ) are listed in Table 2.2. Almost all of the measured adsorption isotherms were classified as L-type (“langmuirian”) according to Giles et al. [54]. The Giles system distinguishes four basic classes of isotherms, depending on the initial slopes. For each group, there are several subgroups depending on the shape of the upper part of the curve.

The “langmuirian” adsorption isotherms of Zn onto soils are illustrated in Fig. 2.8. The adsorption curves were different between the two soils: the curve was nearly L2-type for the humic horizons and intermediate between L1 and C1 Gilles-type for the illuvial horizon. The maximum adsorption capacity  $q_{\max}$  of the humic horizon was 1.5 times higher than that of the illuvial horizon (Table 2.2). This is presumably linked to the high concentration of organic compounds with various functional groups in the organic-rich (humic) soil horizon, capable of

efficiently binding heavy metals [55–57]. Several researchers reported a decrease in the Zn adsorption onto soils after the removal of SOM [10, 50]. In contrast, in the illuvial horizon, the SOM may be physically and chemically immobilized onto the soil clays and protected by mineral pore spaces and fine particles [58].



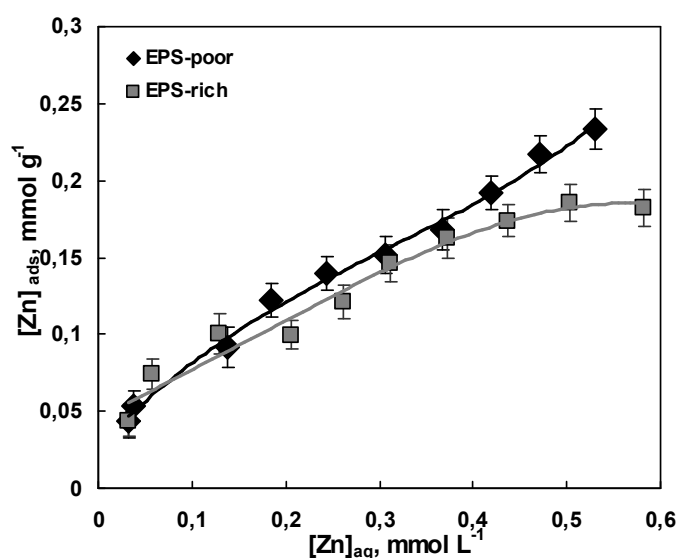
**Fig. 2.8.** Zinc adsorbed onto soil as a function of the metal concentration in the solution at pH  $6.9 \pm 0.1$ . Lines represent the LPM model results.

**Table 2.2.** Langmuir parameters ( $K_L$  and  $q_{max}$ ) computed from the experiments at various aqueous zinc concentrations.

experiment	$K_L, \text{g} \cdot \text{mmol}^{-1}$	$q_{max}, \text{mmol} \cdot \text{g}^{-1}$
EPS-rich	0.003	0.196
EPS-poor	0.004	0.256
humic horizon	0.078	0.128
illuvial horizon	0.005	0.084
humic horizon + EPS-rich ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.081	0.123
humic horizon + EPS-poor ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.085	0.130
humic horizon + EPS-rich ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.058	0.100
humic horizon + EPS-poor ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.062	0.125
illuvial horizon + EPS-rich ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.004	0.046
illuvial horizon + EPS-poor ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.009	0.064
illuvial horizon + EPS-rich ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.004	0.030
illuvial horizon + EPS-poor ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.007	0.038

Zinc adsorption on both the EPS-rich and EPS-poor bacteria surfaces presents similar features for both strains, with L2-type adsorption isotherms (Fig. 2.9). Note however that the EPS-rich strain demonstrates surface-site saturation at lower Zn concentrations in solution compared to that of the EPS-poor strain. This difference between EPS-rich and EPS-poor strain

is statistically significant (at  $p < 0.05$ ) only for Zn concentration above 0.4–0.45 mM. The lower adsorption onto the EPS-rich strain has also been reported for Cu [28], although in the latter case, the difference between the two strains was much higher than that for the Zn in this study. The different affinities of the bacterial cells for Zn and Cu suggest certain discrimination mechanisms among metals and are presumably linked to the much higher toxicity of Cu compared to Zn [cf., 59], as discussed below. The values of  $q_{\max}$  for *P. aureofaciens* obtained in this study (Table 2.2) are comparable with those obtained in experiments of Zn adsorption onto aquatic plants [60–63] but lower than the adsorption parameters of Zn onto mosses [39, 64]. At the same time, the maximal adsorption capacity of Zn onto bacteria at  $\text{pH} = 6.9$  is a factor of 2 and 3 times higher than those on the humic and illuvial soil horizon, respectively. Note that this difference becomes more than an order of magnitude higher after normalization of the adsorption yield to the dry weight of the bacteria instead of the wet weight.

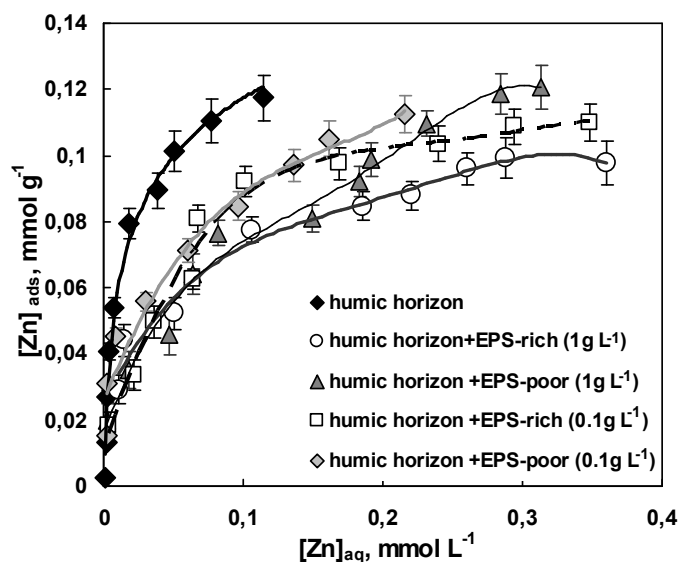


**Fig. 2.9.** Zinc adsorbed onto the surface of *P. aureofaciens* as a function of the metal concentration in the solution at  $\text{pH} 6.9 \pm 0.1$ . Lines represent the LPM model results.

Zn adsorption onto a mixture of the humic soil horizon and bacterial cells at a concentration of  $4 \text{ g}_{\text{dry}} \text{ L}^{-1}$  and  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ , respectively, yielded the maximal adsorption value of  $0.10 \text{ mmol g}^{-1}$  and  $0.125 \text{ mmol g}^{-1}$  for the EPS-rich and EPS-poor strains, respectively (Table 2.2). The Zn adsorption isotherms on both the humic soil horizon and the EPS-rich *P. aureofaciens* belong to the L4-type of the Giles classification (Fig. 2.10). The calculated values

of  $K_L$  for these experiments are similar ( $p < 0.05$ ) to those for Zn adsorption onto the humic soil horizon without bacteria (Table 2.2). This suggests that, during the competition of active adsorption centers for Zn, the soil centers are saturated first, followed by the saturation of the bacterial-surface centers. The Zn adsorption isotherm onto soils in the presence of the EPS-poor *P. aureofaciens* belongs to the transient L2–L4 type. Due to the lower EPS content, this bacterial strain blocks the soil surface centers to a smaller degree compared to the EPS-rich one.

The decrease in the bacterial concentration in the system slightly decreases the adsorption parameters. For example, the  $q_{\max}$  value for Zn onto the humic horizon with a bacterial concentration of  $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$  is slightly higher than that with a concentration of  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$  ( $0.123$  and  $0.143 \text{ mmol g}^{-1}$  for EPS-rich and EPS-poor, respectively, Table 2.2). Although this difference is within the experimental scatter, it is still significant ( $p < 0.05$ ). Presumably, in the former case, due to the lower concentration of bacterial surface centers, the competition between the different sorbents is weak. The adsorption isotherms for  $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$  bacteria experiments belong to the L2-type of Gilles (Fig. 2.10).

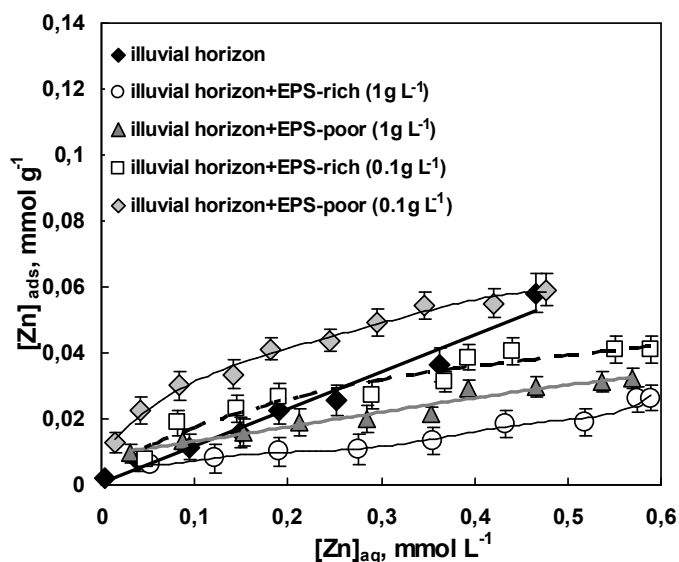


**Fig. 2.10.** Zinc adsorbed onto the humic horizon of podzol and the surface of *P. aureofaciens* as a function of the metal concentration in the solution at  $\text{pH } 6.9 \pm 0.1$ . Lines represent the LPM model results.

The  $q_{\max}$  values in the adsorption system with the illuvial soil horizon ( $4 \text{ g}_{\text{dry}} \text{ L}^{-1}$ ) and bacteria ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ ) are  $0.046$  and  $0.064 \text{ mmol g}^{-1}$  for the EPS-rich and EPS-poor strains,



respectively. The adsorption curves are similar for both strains and are L2-type. In contrast to the humic horizon, during a bacterial concentration decrease in the mineral soil system, the  $q_{\max}$  increased by a factor of 1.5–1.7. Overall, the adsorption of Zn onto the illuvial soil horizon is much less sensitive to the presence of bacteria (Fig. 2.11) compared to the adsorption onto the humic horizon (Fig. 2.10). This is confirmed by rigorous statistical analysis of the adsorption curves. In the case of humic horizon, bacteria-free adsorption is significantly ( $p < 0.05$ ) higher than that in the biotic systems in the full range of studied Zn concentration. For the illuvial horizon, the abiotic adsorption is significantly ( $p < 0.05$ ) higher than that of  $1 \text{ g L}^{-1}$  bacterial experiments only at  $[\text{Zn}]_{\text{aq}} > 0.25 \text{ mM}$ .



**Fig. 2.11.** Zinc adsorbed onto the illuvial horizon of podzol and the surface of *P. aureofaciens* as a function of the metal concentration in the solution at  $\text{pH } 6.9 \pm 0.1$ . Lines represent the LPM model results.

#### 2.3.4. Linear-programming modeling

The results of the LPM treatment of all of the experimental data are listed in Tables 2.3 and 2.4. There are significant differences in the thermodynamic parameters of the Zn adsorption in different systems, and these differences are consistent with the empirical treatment of the adsorption isotherms presented in the previous sections. Experiments on the Zn adsorption as a

function of the solution pH yielded the highest binding site number for the EPS-rich *P. aureofaciens* ( $0.25 \text{ mmol g}^{-1}$ , Table 2.3). In the bacteria-free system, the binding site number of the humic soil horizon is 2.3 times higher than that of the illuvial horizon. The lowest *pKs* values (Table 2.3), corresponding to the strongest Zn binding, were observed for the EPS-poor *P. aureofaciens* (-1.2) and bacteria-free soils (-1.9 and -0.5 for the humic and illuvial horizons, respectively). The presence of bacteria in the humic soil system does not significantly ( $p > 0.05$ ) change the total number of binding sites (from  $2.11 \cdot 10^{-2}$  to  $(1.8\text{--}2.16) \cdot 10^{-2} \text{ mmol g}^{-1}$ ) as it increases the *pKs* value. The addition of  $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$  bacteria to the illuvial soil increases the binding site number from  $0.91 \cdot 10^{-2}$  to  $2.16 \cdot 10^{-2} \text{ mmol g}^{-1}$  and it decreases the value of *pKs*.

**Table 2.3.** LPM parameters for zinc adsorption onto soils: bacteria and soil+bacteria mixtures as a function of the pH.

type of experiments	Binding sites $\cdot 10^{-2}$ , $\text{mmol g}^{-1}$	<i>pKs</i>
humic horizon	2.11	-1.9
illuvial horizon	0.91	-0.5
EPS-poor	0.10; 0.13; 0.90	-1.2; 3.1; 5.0
EPS-rich	25	2.5
humic horizon + EPS-poor ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	2.16	-0.6
humic horizon + EPS-rich ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	1.17 and 0.87	-0.5 and 1.8
humic horizon + EPS-poor ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.23; 0.35; 1.25	0.6; 1.2; 1.7
humic horizon + EPS-rich ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	1.19; 0.39; 0.24	0.65; 1.1; 4.3
illuvial horizon + EPS-poor ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	2.05 and 0.12	-1.0 and 0.8
illuvial horizon + EPS-rich ( $0.1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.73 and 1.37	-0.7 and 0.2
illuvial horizon + EPS-poor ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	0.30 and 1.29	0.8 and 0.9
illuvial horizon + EPS-rich ( $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ )	1.20 and 0.45	1.1 and 1.6

The LPM results of the “langmuirian” adsorption experiments conducted at a constant pH of  $6.9 \pm 0.1$  are listed in Table 2.4. The binding site number of Zn onto the humic horizon of the soil is an order of magnitude higher than that onto the illuvial horizon and onto the surface of the bacteria. An important result in full agreement with the previous observation is that in two-component systems, the lower the bacteria concentration, the higher the total site number for Zn adsorption.

**Table 2.4.** LPM parameters for zinc adsorption as a function of the metal concentration in the solution (“langmuirian” adsorption experiments).

type of experiments	Binding sites, mmol g <sup>-1</sup>	<i>pKm</i>
humic horizon	0.10 and 8.60	-0.01 and 2.10
illuvial horizon	0.01 and 0.17	1.10 and 4.90
EPS-poor	0.16	2.20 and 4.10
EPS-rich	0.05 and 0.54	6.60
humic horizon + EPS-poor (0.1 g <sub>wet</sub> L <sup>-1</sup> )	0.02; 0.10 and 9.0	-0.01; 2.10 and 4.90
humic horizon + EPS-rich (0.1 g <sub>wet</sub> L <sup>-1</sup> )	0.01 and 0.16	-0.01 and 2.20
humic horizon + EPS-poor (1 g <sub>wet</sub> L <sup>-1</sup> )	0.04 and 12.0	0.75 and 4.80
humic horizon + EPS-rich (1 g <sub>wet</sub> L <sup>-1</sup> )	0.03 and 0.10	0.40 and 2.20
illuvial horizon + EPS-poor (0.1 g <sub>wet</sub> L <sup>-1</sup> )	0.10 and 6.54	0.01 and 5.50
illuvial horizon + EPS-rich (0.1 g <sub>wet</sub> L <sup>-1</sup> )	0.05 and 0.45	0.90 and 4.70
illuvial horizon + EPS-poor (1 g <sub>wet</sub> L <sup>-1</sup> )	0.03 and 0.09	1.30 and 3.10
illuvial horizon +EPS-rich (1 g <sub>wet</sub> L <sup>-1</sup> )	0.01 and 0.15	2.40 and 4.40

The lowest *pKm* values were obtained for the humic horizon in the bacteria-free system and with 0.1 g<sub>wet</sub> L<sup>-1</sup> of *P. aureofaciens* (*pKm* = -0.01), whereas the highest *pKm* values were found for the ESP-rich strain (*pKm* = 6.6). The *pKm* values of the soil illuvial horizon and the EPS-poor *P. aureofaciens* are rather similar (1.1, 4.9 and 2.2, 4.1, respectively). The presence of bacteria (0.1 g<sub>wet</sub> L<sup>-1</sup>) in the illuvial soil system increases the binding site number and it decreases the value of *pKm*. The addition of 1 g<sub>wet</sub> L<sup>-1</sup> bacteria does not significantly change the total number of binding sites and increases the *pKm* value.

## 2.4. Conclusions

The experiments on the Zn adsorption onto podzol soil as a function of the pH demonstrated a factor of 1.5 higher sorption capacity for the organic-rich (humic) horizon than for the mineral (illuvial) horizon, presumably linked to the different complexation constants of Zn with the organic and mineral surface functional groups. The Zn adsorption onto the EPS-poor strain of the soil bacterium *P. aureofaciens* is insignificantly ( $p > 0.05$ ) higher than that onto the EPS-rich strain. However, compared to previous results for Cu [28], demonstrating a very strong shielding of the *P. aureofaciens* cell surface sites by the EPS, we observe a much smaller distinction between the EPS-rich and EPS-poor bacteria for Zn because the decrease in the Zn

adsorption on the EPS-rich cultures does not exceed 10% to 20%. This difference in the cell protection between Cu and Zn may be linked to (1) stronger complexation of  $\text{Cu}^{2+}$  relative to  $\text{Zn}^{2+}$  by the surface sites of the EPS-free cells compared to the EPS-rich strain surface; (2) the lack of an active physiological response of the cells to the presence of low  $\text{Zn}^{2+}_{(\text{aq})}$  concentrations; and/or (3) a much higher toxicity threshold for Zn compared to Cu.

The adsorption in the binary soil + bacteria systems exhibits rather conservative behavior during the Zn interaction with the illuvial (mineral) soil horizons in the presence of both the EPS-rich and EPS-poor cultures. In contrast, there is a non-additive effect of the Zn adsorption decrease onto the organic-rich (humic) horizon in the presence of the bacteria. In other words, whereas for the illuvial horizon, the presence of bacteria does not significantly modify the Zn adsorption, the humic, organic-rich horizon exhibits almost a factor of 2 lower adsorption of Zn in the presence of live *P. aureofaciens*. Among the two contrasting bacterial strains, the EPS-rich cells are the most efficient “inhibitors” of Zn adsorption onto soils.

We hypothesize that the main mechanisms of the decrease in the Zn adsorption onto the soil in the presence of the EPS-rich bacteria is shielding/protection of the soil organic-rich particles by the relatively inert EPS. For example, the adsorption of EPS onto soil particles may lead to their aggregation, a decrease of the active surface area and, presumably, screening of the active surface centers from the aqueous  $\text{Zn}^{2+}$  ions. Special transmission electron microscopy and laser granulometry studies of soil–bacteria aggregates are necessary to confirm this mechanism. It follows that the adsorption of bacterial EPS onto SOM particles of the humic horizon is higher than the EPS adsorption onto mineral particles from the illuvial horizon. However, batch adsorption experiments of bacterial EPS onto soil particles are necessary to quantify this possibility.

An important practical conclusion of this study is that bacteria-free soil may adsorb two times more Zn than soil systems with bacteria. For remediation strategies, abiotically treated soils may be more efficient metal adsorbents than the native (untreated) soil systems.

## Acknowledgements:

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## References

- [1] M. Ledin, C. Krantz-Rolcker, B. Allard, *Soil Biol. Biochem.* 28 (6) (1996) 791-799.
- [2] H.B. Bradl, *J. Colloid Interface Sci.* 277 (1) (2004) 1–18.
- [3] J.B. Fein, C.J. Daughney, N. Yee, T. Davis, *Geochim. Cosmochim. Acta* 61 (1997) 3319–3328.
- [4] Yu.V. Yuduntseva, Yu.M. Khodorovskiy, A.G. Zyulikova, S.D. Sokolova, *Soviet Soil Sci.* 14 (3) (1982) 56-62.
- [5] S. Dahiya, A.V. Shanwal, H.A. Hedge, *Chemosphere* 60 (2005) 1253-1261.
- [6] M. Arias, C. Pérez-Novo, F. Osorio, E. López, B. Soto, *J. Colloid Interface Sci.* 288 (2005) 21-29.
- [7] D. Singh, G. Ronald, McLaren, K.C. Cameron, *Soil Sci. Plant Anal.* 39 (2008) 2971-2984.
- [8] M. Zahedifar, N. Karimian, J. Yasrebi, *Comm. Soil Sci. Plant Anal.* 41 (2010) 897-907.
- [9] C. Pérez-Novo, D. Fernández-Calviño, A. Bermúdez-Couso, J.E. López-Periago, M. Arias-Estévez, *Chemosphere.* 83 (7) (2011) 1028–1034.
- [10] C. Pérez-Novo, M. Pateiro-Moure, F. Osorio, J.C. Nóvoa-Muñoz, E. López-Periago, M. Arias-Estévez, *J. Colloid Interface Sci.* 322 (2008) 33–40.
- [11] V. Girija Veni, R.K. Rattan, S.P.Datta, *Int. J. Agricultural Sci.* 3 (9) (2013) 22-25.
- [12] A. Obrador, J. Novillo, M. Alvarez, *Soil Sci. Soc. Am. J.* 67 (2003) 564-572.
- [13] E.R. Orhue, U.O. Frank, *J. Appl. Natural Sci.* 3 (1) (2011) 131-138.
- [14] F.J. Stevenson, *Cycles of soil: Carbon, Nitrogen, Phosphorus, Sulfur, Micronutrients.* New York: Wiley. 1986. p. 380.

- [15] S.M. Barns, S.A. Nierzwicki-Bauer, Microbial diversity in ocean, surface, and subsurface environments. *Geomicrobiology: Interactions Between Microbes and Minerals*, in: Banfield, J.F., Nealson, K.H. Eds., *Reviews in Mineralogy 35* Mineralogical Society of America, Washington, DC, 1997, pp. 35–79.
- [16] J.B. Fein, *Chemical Geology* 169 (2000) 265–280.
- [17] M. Ledin, *Earth-Sci. Reviews* 51 (2000) 1-31.
- [18] B.S. Ausmus, D.R. Jackson, G.J. Dodson, *Pedobiologia* Bd 17 (1977) 183-188.
- [19] R.E. Wildung, T.R. Garland, H. Drucker, Nickel complexes with soil microbial metabolites - mobility and speciation. *ACS Symposium Series 93*, 1979, pp. 181-200.
- [20] P. Chanmugathas, J.M. Bollag, *Soil Sci. Society America J.* 51 (1987) 1184–1191.
- [21] C.J. Daughney, J.B. Fein, N. Yee, *Chem. Geol.* 144 (1998) 151–176.
- [22] D. Borrok, J.B. Fein, *Geochim. Cosmochim. Acta* 68 (14) (2004) 3043–3052.
- [23] K.J. Johnson, J.E.S. Szymanowski, D. Borrok, T. Q. Huynh, J.B. Fein, *Chem. Geol.* 239 (2007) 13–26.
- [24] T.J. Beveridge, R.G.E. Murray, *J. Bacteriol.* 141 (1980) 876–887.
- [25] M. Ueshima, B.R. Ginn, E.A. Haack, J.E.S. Szymanowski, J.B. Fein, *Geochim. Cosmochim. Acta* 72 (24) (2008) 5885–5895.
- [26] T.J. Beveridge, Wall Ultrastructure: How little we know, in *Antibiotic Inhibition of the Bacterial Cell: Surface Assembly and Function*, P. Actor, L. Daneo-Moore, M.L. Higgins, M.R.J. Salton, and G.D. Shockman (ed.). *Amer. Soc. Microbiol.*, Washington, DC, 1988, pp. 3-20.
- [27] P.F.A.M. Römkens, L.A. Bouwman, G.T. Boon, *Environ. Pollut.* 106 (1999) 315-321.
- [28] A.G. González, L.S. Shirokova, O.S. Pokrovsky, E.E. Emnova, R.E. Martínez, J.M. Santana-Casiano, M. González-Dávila, G.S. Pokrovski, *J. Colloid Interface Sci.* 350 (2010) 305–314.

- [29] S.K. Kazy, P. Sar, S.P. Singh, K. Sen Asish, S.F. D'Souza, *World J. Microbiol. Biotechnol.* 18 (6) (2002) 583-588.
- [30] M. Arias, C. Pérez-Novo, E. López, B. Soto, *Geoderma* 133 (2006) 151–159.
- [31] O.S. Pokrovsky, R.E. Martinez, S.V. Golubev, E.I. Kompantseva, L.S. Shirokova, *Appl. Geochem.* 23 (2008) 2574–2588.
- [32] E.B.Ö. Güngör, M. Bekbölet, *Geoderma*, 159 (2010) 131-138.
- [33] J.E. McLean, M.W. Pabst, C.D. Miller, C.O. Dimkpa, A.J. Anderson, *Chemosphere* 91 (2013) 374–382.
- [34] P.G. Wightman, J.B. Fein, *Chem. Geol.* 180 (2001) 55–65.
- [35] Yu.V. Alekhin, O.Yu. Drozdova, Yu.A. Zavgorodnyaya, G.V. Motuzova, *Moscow Univ. Geol. Bul.* 68 (6) (2013) 380–387.
- [36] E.E. Emnova, R.L. Tate, D. Gimenez, A. Dascualinc, S. Toma, O.Gojnetschi, I. Seniscovscaia, in: *II International Symposium: Advanced Biological Technologies and their Impact on Economy “Natural Products: Technologies for their Capitalization in Agriculture, Medicine, and Food Industry”*, 2005, p. 128.
- [37] J.D. Allison, D.S. Brown, K.S. Novo-Gradac, MINTQA2/PRODEFA2, *A Geochemical Assesment Model for Environment Systems. Version 3.0 Users Manual.* U.S. EPA, Athens, GA, 1991.
- [38] O.S. Pokrovsky, R.E. Martinez, E.I. Kompantseva, L.S. Shirokova, *Chem. Geol.* 335 (2013) 75–86.
- [39] A.G. González, O.S. Pokrovsky, *J. Colloid Interface Sci.* 415 (2014) 169–178.
- [40] J.S. Cox, S. Smith, L.A. Warren, F.G. Ferris, *Environ. Sci. Technol.* 33 (1999) 4514–4521.
- [41] I. Sokolov, D.S. Smith, G.S. Henderson, Y.A. Gorby, F.G. Ferris, *Environ. Sci. Technol.* 35 (2001) 341–347.
- [42] R.E. Martinez, D.S. Smith, E. Kulczycki, F.G. Ferris, *J. Colloid Interface Sci.* 253 (2002) 130–139.

- [43] J. Tourney, B.T. Ngwenya, J.W.F. Mosselmans, M. Magennis, *J. Colloid Interface Sci.* 337 (2009) 381–389.
- [44] V.P. Harden, J.O. Harris, *J. Bacteriol.* 65 (1953) 198–202.
- [45] D. Borrok, K. Aumend, J.B. Fein, *Chem. Geol.* 238 (2007) 44–62.
- [46] M.B. McBride, *Adv. Soil Sci.* 10 (1989) 1–56.
- [47] C.P. Huang, E.A. Rhoads, *J. Coll. Interface Sci.* 131(2) (1989) 289–306.
- [48] G. Brümmer, K. G. Tiller, U. Herms, P. M. Clayton, *Geoderma* 31 (4) (1983) 337–354.
- [49] M.B. McBride, J.J. Blasiak, *Soil Sci. Soc. Am. J.* 43 (1979) 866–870.
- [50] J.O. Agbenen, L.A. Olojo, *Geoderma* 119 (2004) 85–95.
- [51] B.T. Ngwenya, *Chemosphere* 67 (2007) 1982–1992.
- [52] M. Loaec, R. Olier, J. Guezennec, *Water Res.* 31 (5) (1997) 1171–1179.
- [53] Motuzova G.V., Barsova N.Yu., *Eurasian Soil Sci.* 45(8) (2012) 761–767.
- [54] C.H. Giles, T.H. MacEwan, N. Nakhwa, D. Smith, *J. Chem. Society* (1960) 3973–3993.
- [55] N. Brady, R. Weil, *The Nature and Properties of Soils*. 13th Edition. Prentice Hall. Upper Saddle River, New Jersey. p. 960, 2002.
- [56] E.F. Covelo, N. Álvarez, M.L. Andrade Couce, F.A. Vega, P. Marcet, *J. Colloid Interface Sci.* 280 (2004) 343–349.
- [57] Y. Li, Q. Yue, B.Gao, *J. Hazard. Mater.* 178 (2010) 455–461.
- [58] P.C. Gomez, M.P.F. Fontes, A.G. Silva, E.S. Mendora, A.R. Netto, *Soil Sci. Soc. Am. J.* 65 (2001) 1115–1121.
- [59] O.S. Pokrovsky, G.S. Pokrovski, L.S. Shirokova, A.G. Gonzalez, E.E. Emnova, A. Feurtet-Mazel, *Geobiology* 10(2) (2012) 130–49.
- [60] O. Keskinan, M. Goksu, A. Yuceer, M. Basibuyuk, C.Forster, *Process Biochem.* 39 (2) (2003) 179–183.
- [61] O. Keskinan, M.Z.L. Goksu, M. Basibuyuk, C.F. Forster, *Bioresource Technol.* 92 (2) (2004) 197–200.

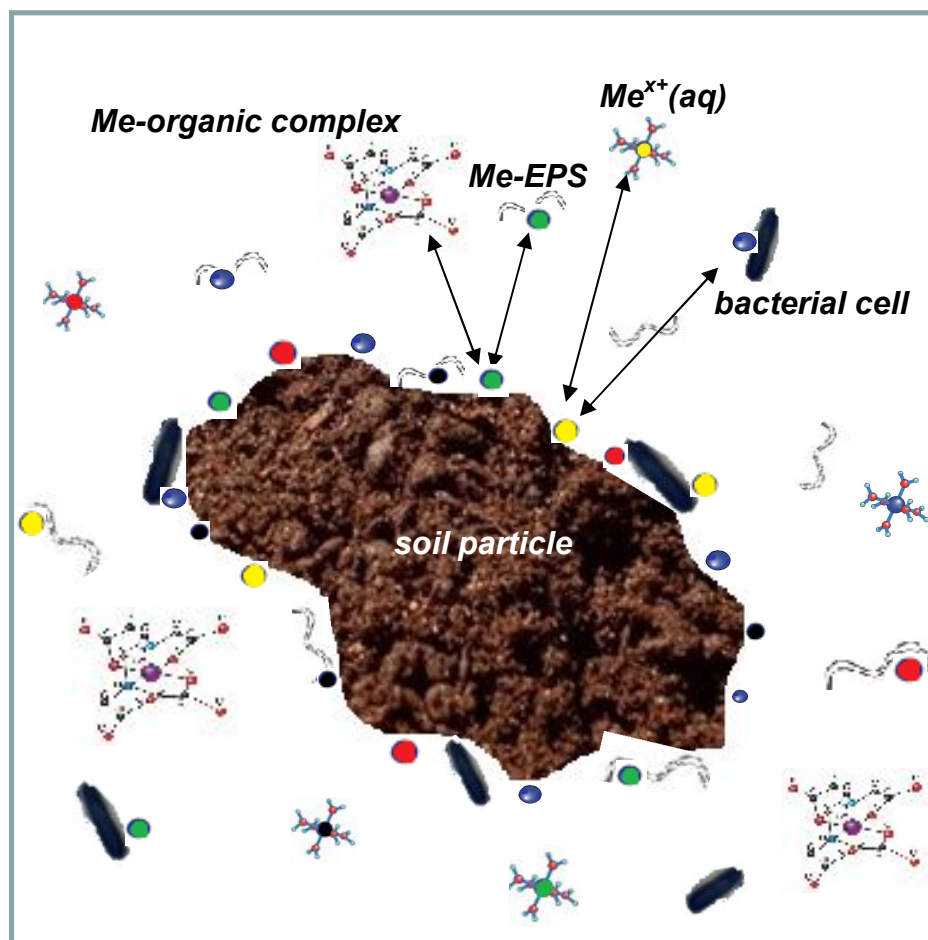


- [62] R.J. Martins, R. Pardo, R.A. Boaventura, *Water Res.* 38 (3) (2004) 693–699.
- [63] Y. P. Kumar, P. King, V.S.R.K. Prasad, *J. Hazard. Mater.* 137 (2006) 1246–1251.
- [64] T. Gosset, J.-L. Trancart, D.R. Thevenot, *Water Res.* 20 (1) (1986) 21–26.

# Chapter 3

## **Impact of heterotrophic bacterium *Pseudomonas aureofaciens* on the release of major and trace elements from podzol soil into aqueous solution**

Chemical geology 410 (2015) 174-187



The processes of weathering and soil formation affect the distribution of metals in the soil liquid phase. There are a lot of mechanisms that control the release of elements in soil. Bacteria can affect on these processes. Several studies have attempted to estimate the effects of bacteria on soil minerals. However, due to the complexities of soil systems, evaluation the mechanisms that control the release of elements in soil is a challenge. Thus, extensive studies on different minerals dissolutions in the presence of bacteria cannot be directly applied to soil environments.

This work aims to quantitatively assess the effects of microbial activity on the leaching of elements from soil in aqueous solution. The main goal of the present study was to reveal the dissolution patterns of major and trace elements for two representative horizons of podzol – organic-rich (eluvial-humic) and mineral (illuvial) in the presence of live and dead bacteria.

# Impact of heterotrophic bacterium *Pseudomonas aureofaciens* on the release of major and trace elements from podzol soil into aqueous solution

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## Abstract

The release of major and trace elements (TEs) from mineral and organic-rich podzol soil horizons has been studied in batch reactors with live and dead soil heterotrophic *Pseudomonas aureofaciens* bacteria and in bacteria-free systems. Dissolved organic carbon (DOC) concentrations decreased and dissolved inorganic carbon concentrations increased over the course of experiments with live bacteria due to on-going DOC mineralization processes. Several families of major and trace elements could be distinguished, depending on their release patterns in bacteria-free and live bacteria-bearing systems. Live bacteria enhanced the release of Mg, Rb, Cd, Pb, Al, Fe and V from the mineral soil horizon and the release of Rb, Ni, Pb, As, Fe, V and La from the organic soil horizon relative to bacteria-free soil or dead bacteria experiments. Unexpectedly, K, Ca, Sr, Cu, Ti, Mn, Zn and As release from the mineral horizon and Mg, K, Ca, Sr, Ba, Cr, Ti, Mn and Zn release from the organic horizon decreased in the presence of live *P. aureofaciens* compared to bacteria-free and dead bacteria systems. Finally, live bacteria exhibited no effect on the release of Si, Al, Cu and Mo from the humic horizon and Ni, Mo, Cr, Ba, Si and La from the mineral horizon relative to the bacteria-free system. These results can be

interpreted via a combination of several simultaneous processes, which occur in the soil-bacteria suspension and lead to changes in TE speciation and affinities to mineral surfaces and bacteria and include the following: 1) a slight decrease in the pH due to exometabolite production; 2) degradation of DOC and TE organic complexes by heterotrophic bacteria; 3) element adsorption at cell surfaces and biological uptake; and 4) element release from the soil mineral and organic particles.

Compared to abiotic systems, the observed decreases in the concentrations of major elements and many heavy metals that leach from the soil in the presence of bacteria have important consequences regarding our understanding of the role of bacteria in element mobilizations from soil to rivers. It follows that chemical weathering in both organic and mineral horizons of podzol soil may not be strongly affected by heterotrophic bacterial activity; rather, the solution pH and DOC levels may control the intensity of element mobilization. The aqueous concentrations of many TEs (Fe, Al, La, Cr, Ni, Cd, Pb, Cu, and Rb) at the end of live-bacteria experiments were comparable with reported compositions of interstitial podzol soil solutions.

**Keywords:** *humic, mineral, soil, metal, bacteria, kinetics*

### **3.1. Introduction**

Among the factors controlling the intensities of chemical weathering in soils, solution pH,  $p\text{CO}_2$  and organic compounds produced during enzymatic degradation of vegetation litter or the exometabolism of soil bacteria and roots are believed to be the most important (Barker et al., 1997; Bennett et al., 2001). In contrast to our rather detailed knowledge regarding the effects of various organic ligands on soil mineral dissolution kinetics, the roles of live bacteria on the release of elements from soils, especially from organic-rich horizons, remain poorly understood. The present work aims to quantitatively assess the effects of microbial activity on the leaching of elements from contrasting soil horizons in aqueous solution. Several studies have attempted to

quantify the effects of bacteria on rock dissolutions (Fein et al., 1999; Wu et al., 2007, 2008) as well as on primary soil minerals (Lee and Fein, 2000; Pokrovsky et al., 2009; Shirokova et al., 2012; Stockmann et al., 2012). However, due to the complexities of whole soil systems, assessing the elementary mechanisms that control the release of elements in mineral – bacteria systems remains a challenge. Thus, given the inherent complexities of these systems and various reaction mechanisms involved in these interactions, extensive studies on aluminosilicate (Barker et al., 1997; Bennett et al., 2001), calcite (Friis et al., 2003; Lüttge and Conrad, 2004; Davis et al., 2007), apatite (Hutchens et al., 2006; Feng et al., 2011) and basalt (Sujith et al., 2014) dissolutions in the presence of bacteria cannot be directly applied to soil environments.

The use of individual soil horizons with reference cultures of soil bacteria offers the possibility of testing, for the first time, the direct effects of live and dead bacteria on the release of major and trace elements from soil matrices. For this, we have chosen typical rhizospheric soil bacterium *Pseudomonas aureofaciens*, which has been relatively well studied with respect to metal adsorption and is known to produce exopolysaccharides (EPS), depending on the type of substrate used for growth (Pokrovsky et al., 2008, 2009; González et al., 2010). The working hypothesis assumes that the presence of live bacteria will enhance the release of both nutrients and indifferent major and trace elements from the soil to aqueous solutions based on modifications to the environment (pH, DOC, nutrient request). Among the most important factors controlling element release during biodegradation of oxyhydroxide-associated organic matter (i.e., Eusterhues et al., 2014), one may anticipate pH and [DOC] changes together with bacterial mineralization of soluble humic and fulvic compounds. The elements may be adsorbed on the bacterial surface, intracellularly assimilated, and, in some cases, complexed by organic ligands of bacterial metabolism (Ledin, 2000), the combination of which is likely to create a complex pattern of element concentration dependence on time. The main goal of the present study was to reveal the dissolution patterns of major and trace elements for two representative horizons of boreal podzols – humic and mineral (illuvial) layers. Specifically, we aimed at

quantifying the effects of live heterotrophic bacteria on major and TE release rates and soil-fluid release ratios. Via rigorous comparisons of live, dead and bacteria-free soil systems, we were able to demonstrate the relative importance of physico-chemical and biological factors on major and TE release from the soil, and we foresee that the results obtained will enable quantitative prediction of interstitial soil solution chemistry and soil mineral weathering intensity under biological control.

## **3.2. Materials and methods**

### *3.2.1. Soils, bacteria, experiments and analyses*

Podzol soil samples of humic (surface) horizon and illuvial (mineral) horizon were collected in the Northern Karelia, which is within the European boreal zone (N 66°18', E 30°42') from the catchment Vostochny (Ilina et al., 2014). The soils were air-dried and autoclaved at 121 °C for 2 h prior to the experiment. The sterility of autoclaved soils was verified by inoculation on nutrient agar; no detectable growth of bacteria was observed. The main physical and chemical properties of soils considered in this study are presented in Table 3.1. The organic carbon content of soils was determined using an Element Analyzer (Vario EL III). Major and trace elements in soils were determined using ICP-MS (Agilent 7500ce series) after complete acid digestion of the sample, as described elsewhere (Viers et al., 2013; Stepanova et al., 2015).

The bacterial strain of soil aerobic gram-negative bacteria *Pseudomonas aureofaciens* CNMN PsB-03 was obtained from the laboratory of Plant Mineral Nutrition and Hydric Regime (Institute of Plant Genetics and Physiology, Moldovan Academy of Sciences, Chisinau, Moldova). The specific strain was isolated from soybean root-adhering (rhizospheric) temperate soil for its ability to produce large amounts of gel-forming exopolysaccharide (EPS) on a sucrose-peptone (SP) medium (Emnova et al., 2005). This bacterial strain was selected due to its

model and well-known surface properties in terms of its interactions with heavy metals and minerals (Pokrovsky et al., 2008, 2009; 2012a).

Cultivation was performed at 25°C for 72 h in liquid SP media (sucrose-peptone solution, pH 7.0), thus promoting rich EPS synthesis (González et al., 2010), with continuous shaking until the stationary growth phase. Investigations of soil – bacteria – aqueous solution interactions were conducted using rinsed bacterial biomass, which was produced by repeated centrifugation (4 times) in an inert electrolyte solution of 0.01 M NaNO<sub>3</sub>. Note that the EPS synthesis by *P. aureofaciens* is significantly smaller in the absence of sucrose-peptone media used in the experiments. Live cell numbering was performed via nutrient agar-plate culturing in triplicates with an average uncertainty of 20%. Dead bacteria biomass was produced by autoclaving rinsed cells at 121°C for 20 min. According to optical observations using immersion microscope, this treatment did not cause significant cell damages and lysis; the cell walls remained intact although the amount of the EPS attached to the cell surface decreased significantly.

Kinetic soil-leaching experiments were conducted at  $23 \pm 2^\circ\text{C}$  in continuously agitated aqueous suspensions of 0.005 M NaNO<sub>3</sub> + soil + bacteria. To avoid any complexation of released metals with solution constituents, no buffer was used. As such, the bacteria were alive during the biotic experiments, but no significant growth could occur. The soil concentration in the experiments was 1 g<sub>dry</sub> L<sup>-1</sup>, and the biomass of bacteria was 1 g<sub>wet</sub> L<sup>-1</sup>. Aliquots of the vigorously shaken suspension were collected after 1, 24, 48, 72, 96, 120, 144 and 168 h and then centrifuged at 4500g, and the resulting supernatant was filtered through a 0.45-µm acetate cellulose filter, acidified with bi-distilled HNO<sub>3</sub> and stored in the refrigerator until analysis. Live, freshly harvested cells and dead (autoclaved) bacteria were used in the experiments. Each experiment was run in triplicate. All filtered solutions were analyzed for aqueous metal concentration using ICP-MS (Agilent 7500ce series) with a detection limit of 0.001 µg L<sup>-1</sup> and a precision of  $\pm 5\%$ . Dissolved organic and inorganic carbon (DOC and DIC) concentrations in non-acidified filtered



solution were measured using a Carbon Total Analyzer (Shimadzu TOC-6000) with an uncertainty of 3% and a detection limit of 0.1 mg L<sup>-1</sup>.

Note that suspension of soils with bacteria used in this study is not capable to mimic directly soil/bacterial interactions in real systems because the normal mode of bacterial life in such systems is the biofilm. However, via simplifying the mode of bacteria-soil interaction, we could identify the patterns of element release and to test biological and physico-chemical rate-controlling factors. For this and in order to increase the resolution, one has to use high fluid/soil ratio most sensitive to fine changes in the fluid composition in response to dissolution of soil grains, element desorption and organic colloids degradation.

### *3.2.2. Data treatment and statistics*

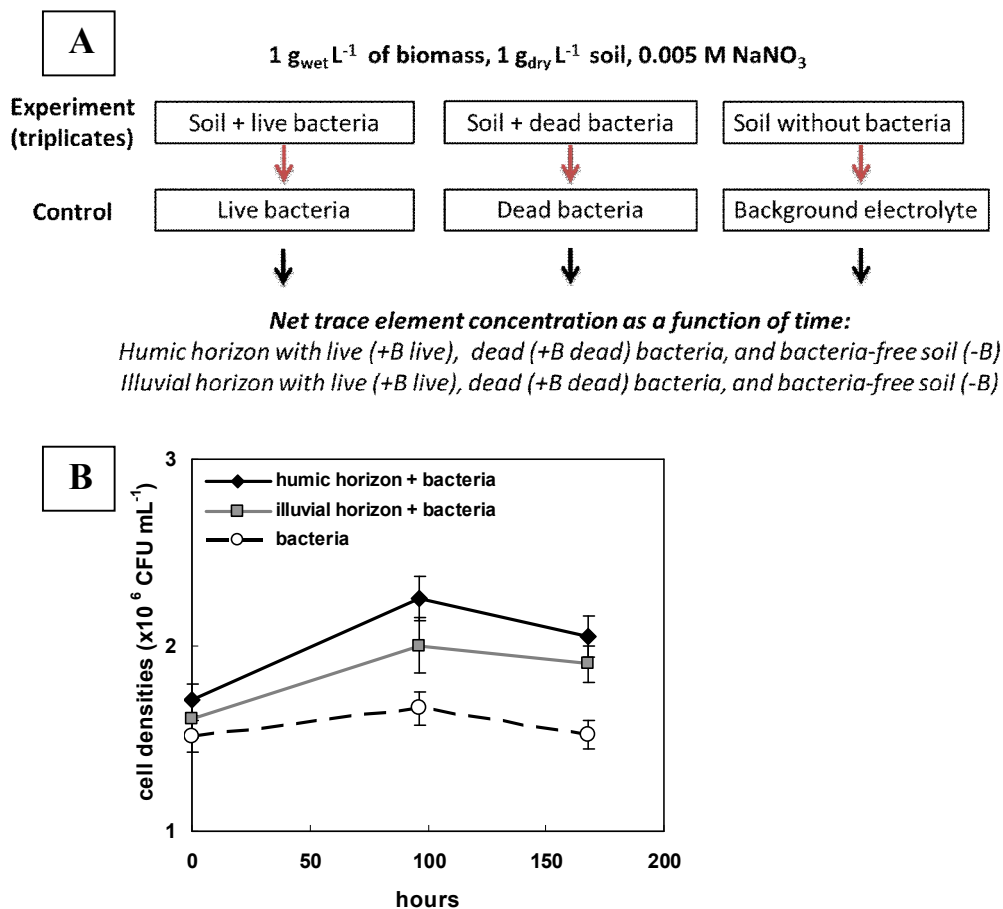
The scheme of conducted experiments is illustrated in Fig. 3.1A. Three main types of experiments included two different soil horizons with live bacteria, with dead bacteria and without bacteria, each in triplicates. The replicate-averaged concentration as a function of time was corrected for element concentration in the control experiments. For this, two types of bacterial control experiments were performed: live bacteria without soil and dead bacteria without soil. Bacteria-free experiment with soil was corrected for element concentration in the background electrolyte.

Because soil is a complex mixture of mineral and organic phases, different TEs may reside in different pools, and the same element may be present in several mineral and organic compounds. Therefore, multiple sources are capable of releasing TEs into aqueous solution. The linear model of first-order reactions provides information with physicochemical meaning and allows us to fit the kinetic data. This model assumes that there are multiple and simultaneous first-order reactions, the rates of which are independent of each other (Baranimotlagh and Gholami, 2013). This is compatible with multicomponent systems like soil, the constituents of

which (minerals, organic matter (OM) debris, humic and fulvic compounds etc.) dissolve in solution independently, each with its own dissolution rate:

$$Q = kt + b, \quad (1)$$

where  $Q$  are the amounts of metals released from the soil into the solution at time  $t$ ,  $k$  is the pseudo first-order rate constant normalized to the soil mass, and  $b$  is the initial mass of trace metal present at the first contact of soil with aqueous solution.



**Fig. 3.1.** Scheme of experiments performed in this study (A) and experimental live bacterial cell densities as a function of time (B).

The steady-state release ratios of major and trace elements (TEs) between the aqueous solution and the soil is calculated as  $R_R = [\text{TE}]_{\text{solution}}/[\text{TE}]_{\text{soil}}$ , where  $[\text{TE}]_{\text{solution}}$  was the concentration of an element released into solution, and  $[\text{TE}]_{\text{soil}}$ , the concentration of this element in the whole soil sample. This element release ratio ( $R_R$ , e.g. Shibata et al., 2006) is thus defined from at least two last data points from six independent experiments corresponding to the pseudo-

equilibrium of soil with an aqueous solution (flattening of the [TE] dependence on elapsed time). This pseudo-equilibrium was assumed to exist if the difference between two last data points was smaller than the experimental reproducibility of their triplicates (2 s.d.).

We used the geochemical program Visual MINTEQ (Gustafsson, 1999), version 3.1 (October 2014), in conjunction with a database and the NICA-Donnan humic ion binding model (Benedetti et al., 1995; Milne et al., 2003) to calculate the speciation of dissolved metals and saturation state of solution with respect to solid phases. Elemental-concentration evolution with time was analyzed using best-fit functions based on the least squares method, Pearson correlation and one-way ANOVA with STATISTICA version 8 software (StatSoft Inc., Tulsa, OK). Statistical treatment of the data also included the use of Microsoft Excel for calculating the average (2 s.d.) of three replicate experiments. Regressions and power functions were used to examine the relationships between elemental concentrations and elapsed time. Correlation coefficients were calculated to elucidate the relationships between the different components of the aqueous solution during the experiment and between aqueous concentrations of elements over time.

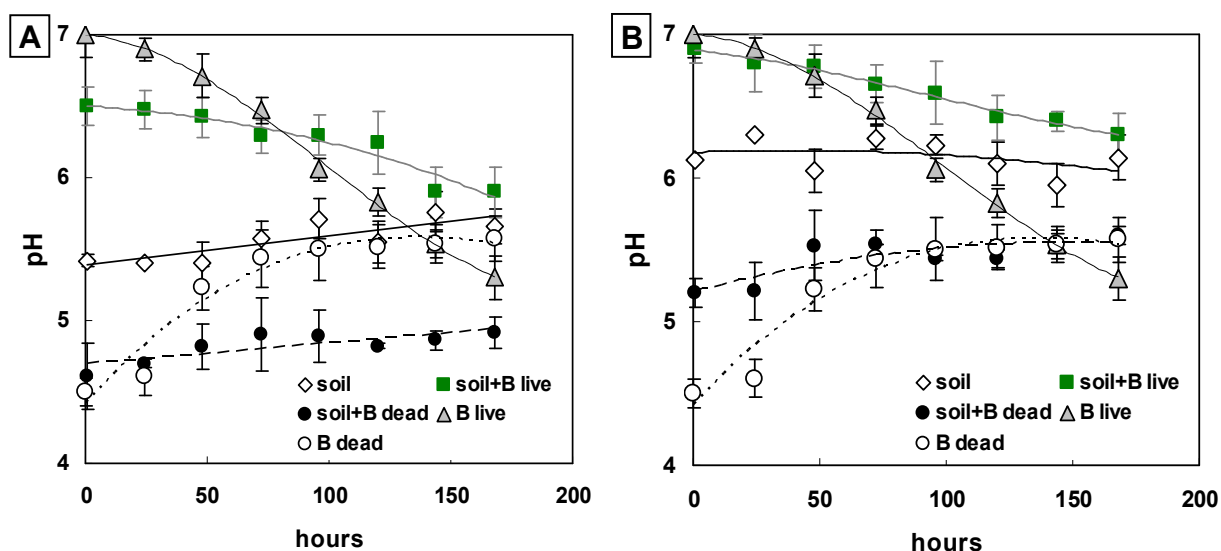
### **3.3. Results**

#### *3.3.1. Bacteria, pH, DOC and DIC concentration*

The live cell concentration was routinely followed in the course of experiments with live and dead bacteria in the presence of soils but also in soil-free system. Over ~170 h of exposure, live bacteria numbers ranged from  $1.75 \cdot 10^6$  to  $2.25 \cdot 10^6$  and from  $1.5 \cdot 10^6$  to  $2.0 \cdot 10^6$  CFU/mL for experiments with humic and illuvial horizons, respectively (Fig. 3.1B). It can be seen that the concentration slightly increased in the presence of soil, and that humic horizon was more beneficial for bacterial growth and maintenance than the mineral horizon. Therefore, the cells remained alive capable producing and consuming dissolved organic matter. In addition to live cell count by agar plate technique, during each sampling, cell concentrations in the suspensions

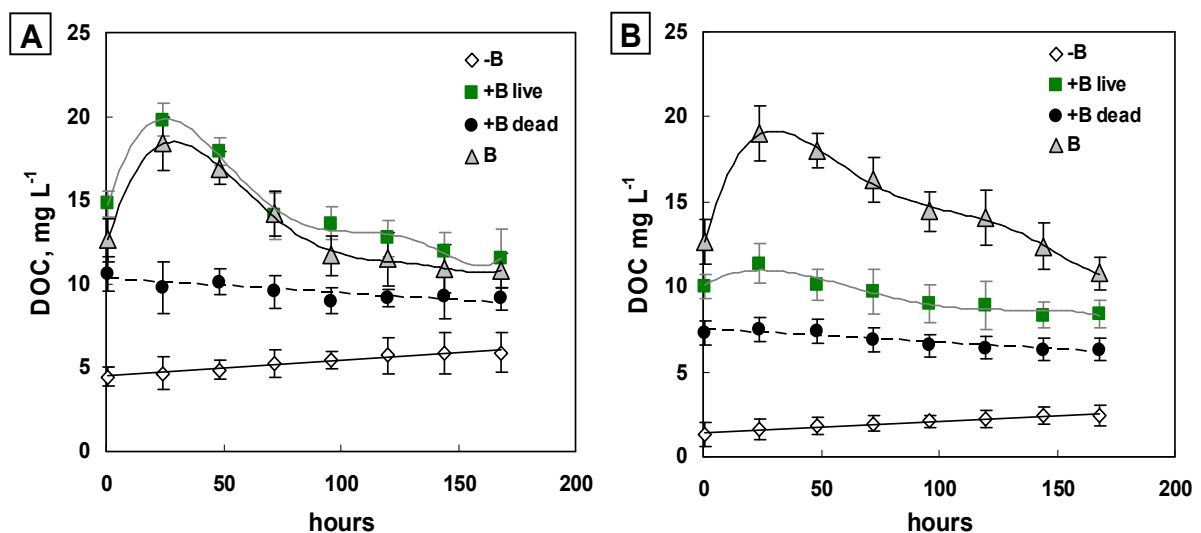
were monitored spectrophotometrically using a 1-cm quartz cell and a wavelength of 600 nm. The variations of the optical densities of the suspensions and, consequently, the biomass of the bacteria, were within  $\pm 10\%$  during the experiment. Examination by optical immersion microscope of the bacterial suspension at the end of each experiment demonstrated that the cells remained intact and without deformation.

All experiments with humic soil horizons yielded lower pH values lower than experiments with illuvial (mineral) horizons (Fig. 3.2). Bacteria-free and dead-bacteria experiments exhibited small pH changes over time, producing, during 160 h of reactions, an increase of 0.24 and 0.3 units, respectively (Fig. 3.2). In contrast, live bacteria produced a gradual decrease in pH up to 0.7 and 0.6 over the course of experiments in humic and mineral soil horizons, respectively. In soil-free system experiments with live bacteria, the drift was between 1.5 and 2 pH units and is most likely linked to the production of acidic exometabolites by live cells (Wu et al., 2006). During the experiments with soils, pH values remained in the circumneutral range (5.5 to 6.5), similar to the values obtained in cell cultures, making cell lysis unlikely (e.g., Ngwenya et al., 2003; Ngwenya, 2007).



**Fig. 3.2.** pH values measured during experiments with humic (A) and illuvial (B) soil horizons. The soil concentration is equal to  $1 \text{ g}_{\text{dry}} \text{ L}^{-1}$ , and the biomass of bacteria is  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ . The legend abbreviation here and in the figures below is as follows: open diamonds, -B: bacteria-free conditions; green squares, +B: live bacteria conditions; and black squares, +B: dead bacteria conditions. The error bars correspond to 2 s.d. of 3 replicates and the lines are for guiding purposes.

Fig. 3.3 shows the DOC concentrations determined in soil-bacteria experiments as a function of elapsed time. The concentrations were systematically higher in experiments with live and dead bacteria compared to bacteria-free soil systems. In the latter case, DOC concentrations were approximately double in organic soil horizons compared to mineral soil horizons. DOC concentrations increased with time in abiotic experiments. This may be related to humic and fulvic acid leaching from soil grains. DOC variations in experiments with dead bacteria were small (within 10%). For both soil horizons, the most significant evolution of DOC occurred in the presence of live bacteria. During the first 24 h of each experiment, there was a 10-25% increase in DOC, an observation that is presumably linked to EPS and other exometabolites produced by live cells. This increase was followed by a decrease in DOC over time, corresponding to heterotrophic consumption of soluble organic matter (i.e., Asmala et al., 2014). Some adsorption of bacterial lysis products and EPS on soil grains cannot be excluded, given the weak decrease in DOC that also occurs in experiments with dead bacteria.



**Fig. 3.3.** Dissolved Organic Carbon measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

Dissolved inorganic carbon (DIC) remained constant for both bacteria-free and dead-bacteria abiotic experiments (Fig. ESM-1). In live cells, there were notable increases in DIC (by a factor of 1.5) during the course of each experiment, which corresponds to cell respiration of

dissolved OM and intracellular organic stocks. Note that the redox measurements of mixed, aerated live cell suspension with submerged O<sub>2</sub> sensor always demonstrated 90±10% O<sub>2</sub> saturation at ambient temperature.

### 3.3.2. Released concentrations as a function of time and element classification

Control experiments (live bacteria or dead bacteria in 0.005 M NaNO<sub>3</sub> without soil) did not show any significant evolution of all TE concentration as a function of time (relative variations are within ±10%). Compared to experiments with soil, corrections for element release in control experiments did not exceed 20% for all major and trace elements discussed below. Elements that required more than 30% correction of their concentration in soil experiments relative to the control and elements that exhibited more than 30% relative variation in the control experiments in the course of time are not presented in this study. In all the graphs shown below the concentration of an element at each sampling time represents the difference between its concentration in soil-bearing experiments and its concentration in the control (soil-free, bacteria-bearing experiments or the background electrolyte).

Analysis of element concentration released as a function of elapsed time demonstrated a common shape of dependencies for most elements: an initial, fast or steady release followed by attenuation (flattening) of the curve [TE] – time (Figs. 3.5-3.12, ESM 2-13). This pattern strongly suggests an initial release that comprised desorption from the soil surface and dissolution of the bulk of soil particles followed by achievement of some stable, steady-state concentration corresponding to a pseudo-equilibrium. The exceptions from this general pattern were Si in both soils for all treatments (Fig. 3.4), Ni, P, Fe, Al, Cd and Pb in humic soil with live bacteria (Fig. 3.8 - 11, ESM-8). For these experiments, no apparent  $R_R$  value could be proposed, although the release rate of all elements was quantified.

The concentrations of most elements were higher in the mineral horizon compared to the organic horizon of podzol soil. Indeed, the majority of metal (Fe, Al) oxyhydroxides are known

to accumulate in the mineral horizon, thereby increasing the concentrations of other trace elements in adsorbed or co-precipitated forms. Depending on their release pattern in bacteria-free and live bacteria systems, several families of major and trace elements could be distinguished. Live bacteria enhanced Mg, Rb, Cd, Pb, Al, Fe and V release from the mineral soil horizon and Rb, Ni, Pb, As, Fe, V and La release from the organic soil horizon. Decreases in release were observed in the presence of live *P. aureofaciens* compared to bacteria-free and dead-bacteria systems for K, Ca, Sr, Cu, Ti, Mn, Zn and As from the mineral horizon and Mg, K, Ca, Sr, Ba, Cr, Ti, Mn and Zn from the organic horizon. Finally, live bacteria exhibited no effects on Si, Al, Cu and Mo release from the humic horizon and Ni, Mo, Cr, Ba, Fe, Si and La from the mineral horizon. The sections below describe element behavior during soil – bacteria interaction for 4 dominant families of major and trace elements. These elements are distinguished by their i) physico-chemical properties in aqueous solutions, determining their speciation and degree of complexation with organic ligands, originated from soil humus or cell metabolism; ii) affinity to live cells and iii) association with organo-mineral colloids. Silica and alkaline-earth metals are present in inorganic forms (neutral molecule and cations) and unlikely to be affected by organic matter complexation or cellular uptake; divalent metals may act as micronutrients (Cu, Mn, Zn, Ni) or toxicants (Cd, Pb); oxyanions and P were present as essentially inorganic species; and finally, trivalent and tetravalent hydrolysates exhibited high affinity to organo-ferric, organo-aluminum non-bioavailable colloids.

#### 3.3.2.1. Silica and alkaline/alkaline-earth elements (Si, Mg, K, Ca, Sr, Ba, Rb)

Compared to abiotic systems, the presence of live bacteria did not significantly modify the release rates of Si in both soil horizons (Fig. 3.4 and Table 3.2). Given the intrinsic scatter of the data and uncertainties of the replicates, the differences observed between the biotic and abiotic treatments are within the limit of experimental resolution and not statistically significant ( $p > 0.05$ ).

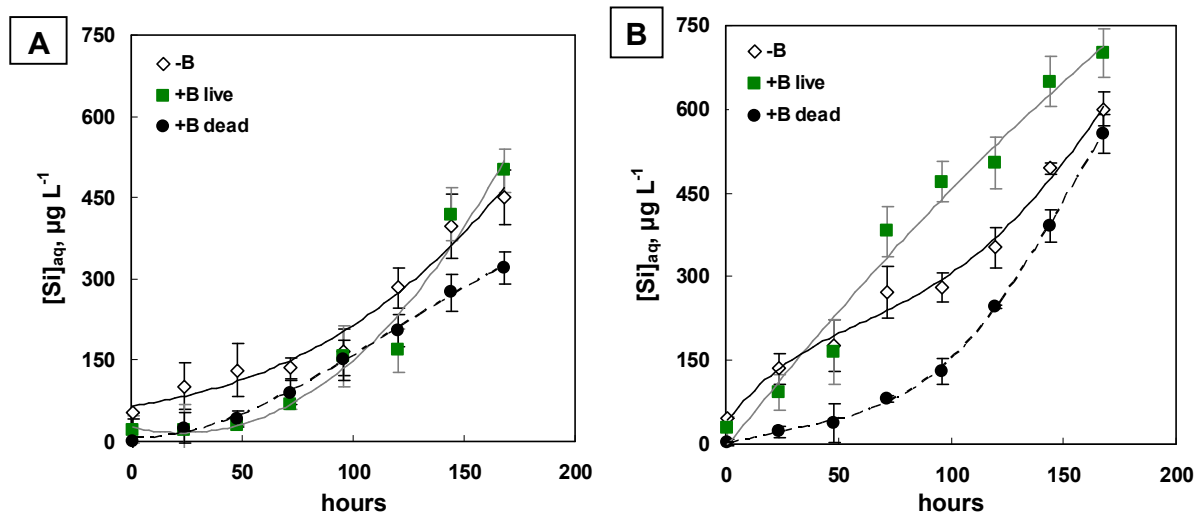
**Table 3.2.** Steady-state  $R_R$  values measured at the end of experiments and pseudo first-order rate constant ( $k$ ,  $\mu\text{g L}^{-1} \text{h}^{-1} \text{g}^{-1}$ , **Eqn. 1**) for the model fit to the data.

		humic horizon		illuvial horizon	
		$R_R * 10^6$	$k$	$R_R * 10^6$	$k$
<b>Mg</b>	-B	9.76	0.21	5.45	0.22
	+B live	6.49	0.06	19.7	0.30
	+B dead	6.04	N.D.	0.79	N.D.
<b>K</b>	-B	4.31	0.35	7.41	0.63
	+B live	N.A.	1.05	20.9	0.64
	+B dead	8.8	0.38	6.00	0.001
<b>Ca</b>	-B	12.5	0.47	11.0	0.51
	+B live	-32.6	-0.55	14.6	0.40
	+B dead	71.2	0.30	-5.05	-0.12
<b>Rb</b>	-B	4.38	0.001	9.89	0.003
	+B live	11.4	0.003	31.3	0.01
	+B dead	3.79	N.D.	1.81	N.D.
<b>Sr</b>	-B	9.51	0.007	11.2	0.005
	+B live	8.61	0.002	9.54	0.001
	+B dead	7.35	N.D.	8.83	0.0005
<b>Ba</b>	-B	4.57	0.012	7.49	0.02
	+B live	0.39	0.002	20.9	0.02
	+B dead	0.31	N.D.	1.18	N.D.
<b>Al</b>	-B	N.A.	0.42	14.8	5.22
	+B live	N.A.	0.59	20.8	6.86
	+B dead	0.15	N.D.	0.82	0.10
<b>Si</b>	-B	N.A.	2.36	N.A.	3.09
	+B live	N.A.	2.91	N.A.	4.28
	+B dead	N.A.	2.00	N.A.	3.18
<b>P</b>	-B	123	0.02	17.5	0.03
	+B live	N.A.	1.97	-469	-0.05
	+B dead	143	0.04	59.5	N.D.
<b>As</b>	-B	0.29	0.0002	0.22	0.0001
	+B live	0.96	0.0004	0.24	0.00001
	+B dead	0.19	N.D.	0.12	0.0001
<b>Ti</b>	-B	1.84	0.02	0.13	0.001
	+B live	2.21	0.007	0.13	0.001
	+B dead	0.2	0.002	0.16	0.001
<b>V</b>	-B	4.25	0.001	1.57	0.0003
	+B live	19.2	0.003	26.7	0.01
	+B dead	-3.77	N.D.	-1.12	N.D.
<b>Cr</b>	-B	5.51	0.001	11.1	0.003
	+B live	N.A.	0.001	12.3	0.004
	+B dead	5.49	N.D.	4.81	N.D.
<b>Mn</b>	-B	8.12	0.006	11.4	0.01
	+B live	2.19	0.002	4.22	0.004
	+B dead	2.96	0.001	1.46	N.D.
<b>Fe</b>	-B	1.06	0.11	18.9	2.62
	+B live	N.A.	0.71	26.9	3.99
	+B dead	0.01	N.D.	0.02	N.D.
<b>Ni</b>	-B	12.1	0.0007	16.1	0.002
	+B live	92.2	0.005	47.1	0.005
	+B dead	3.57	0.0002	5.93	0.00008
<b>Cu</b>	-B	150	0.001	118	0.003
	+B live	74.9	0.0005	157	0.002
	+B dead	47.2	N.D.	20.7	N.D.



	humic horizon		illuvial horizon		
	$R_R * 10^6$	$k$	$R_R * 10^6$	$k$	
<b>Zn</b>	-B	40,6	0.003	34,8	0.005
	+B live	55.5	0.001	26.0	0.002
	+B dead	33.0	0.001	11.7	0.0007
<b>Mo</b>	-B	682	0.0002	134	0.0002
	+B live	4882	0.0003	51.8	0.0002
	+B dead	951	0.0002	16.8	0.00003
<b>Cd</b>	-B	31.0	0.00001	238	0.0001
	+B live	124	0.00005	898	0.0005
	+B dead	-2.43	N.D.	18.6	N.D.
<b>Pb</b>	-B	3.03	0.0002	2.35	0.0001
	+B live	N.A.	0.004	16.5	0.0006
	+B dead	0.14	N.D.	2.95	N.D.
<b>La</b>	-B	3.80	0.0001	15.9	0.001
	+B live	60.7	0.0005	39.7	0.001
	+B dead	1.77	N.D.	0.42	N.D.

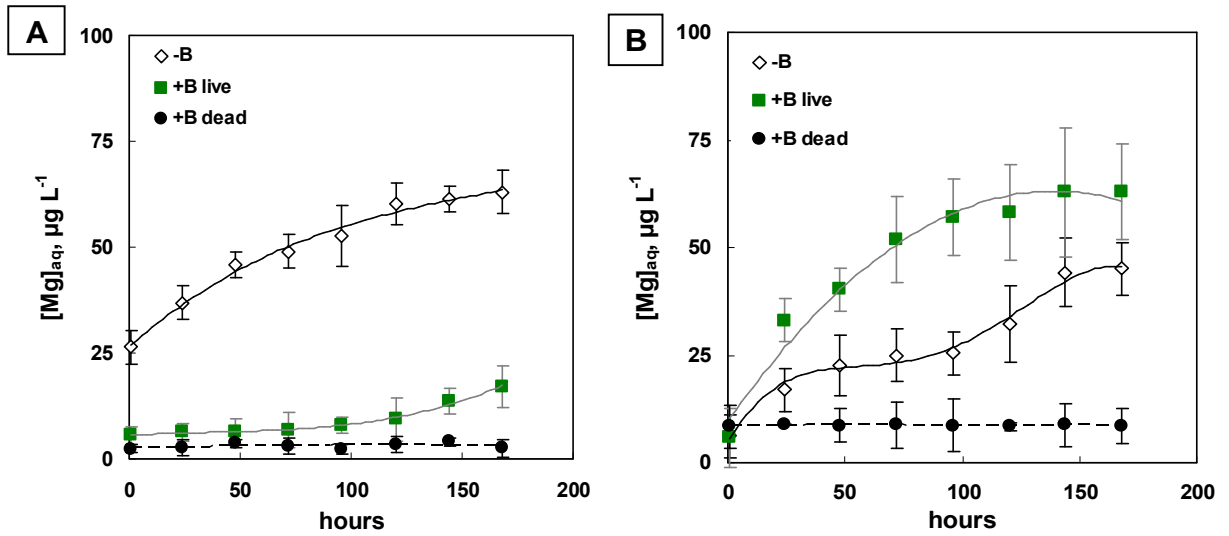
N.D. <0.000001. Negative values indicate that the concentration of element decreased, not increase during the experiment. N.A. for  $R_R$  means the lack of steady-state concentration at the end of experiment.



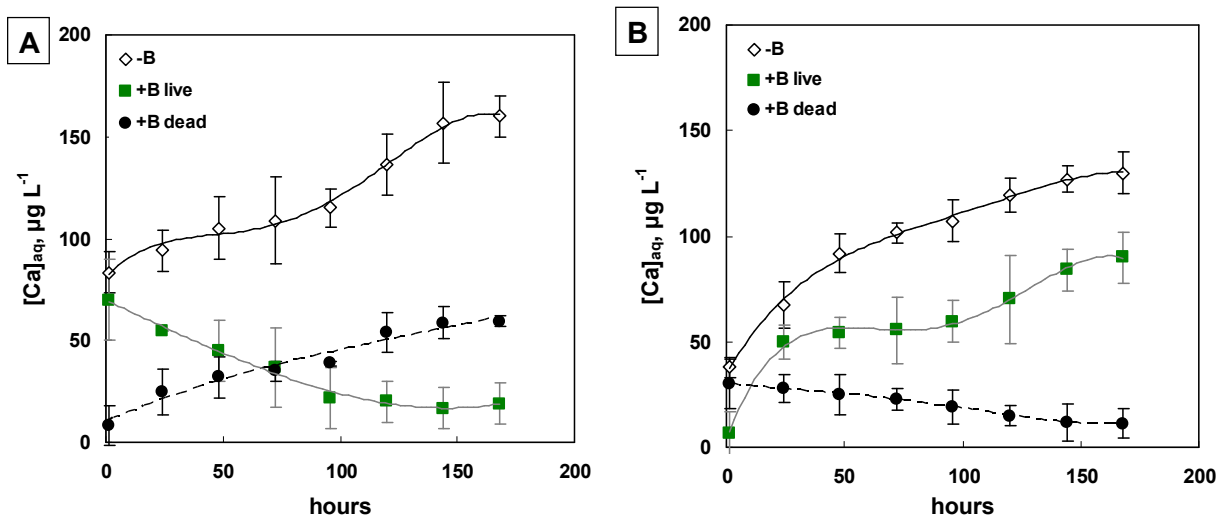
**Fig. 3.4.** Concentration of Si measured during experiments in humic (A) and illuvial (B) horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2. Here and in all figures below, measured element concentrations in “+B live” and “+B dead” series were corrected for the amount of element released from live or dead bacteria in control experiments (see Fig. 3.1).

Addition of live bacteria decreased release rates of Mg and Ca from the humic horizon, whereas releases in mineral soil remained virtually the same in live-bacteria and bacteria-free experiments (Figs. 3.5 and 3.6, respectively), consistent with apparent kinetic constants (Table

3.2). Experiments using the humic soil horizon demonstrated a decrease in Ca concentration with time in the presence of live bacteria (Fig. 3.6 A).



**Fig. 3.5.** Concentration of Mg measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.



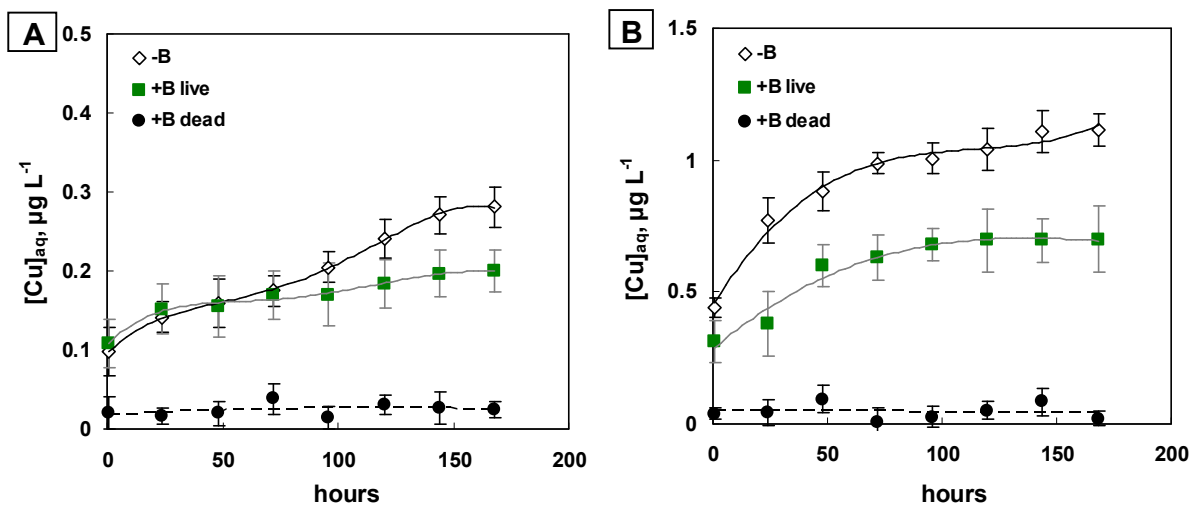
**Fig. 3.6.** Concentration of Ca measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

Sr and Ba exhibited generally similar behaviors, with bacteria-free systems exhibiting maximal rates and release ratios (Fig. ESM-2, 3). An exception was Ba in the mineral horizon, where the rates were similar between bacteria-free and live bacteria treatments (Fig. ESM-3 B). Note that, according to vMinteq calculation, solutions were strongly undersaturated with respect to alkaline earth carbonates. In all treatments (except dead bacteria in the mineral horizon), potassium concentrations increased over time, with live-bacteria experiments in humic soil

exhibiting the highest release rate constants and  $R_R$  values. Live bacteria enhanced K release from organic soil relative to control but had no significant effect on K release in the mineral horizon relative to bacteria-free soil (Fig. ESM-4, Table 3.2). Rubidium behavior followed that of K in humic horizon but contrasted with K behavior in mineral horizon, with the former showing rate-constant increases, by factors of 3 to 4, in live-bacteria treatments compared to bacteria-free experiments (Fig. ESM-5).

### 3.3.2.2. Divalent heavy metals (Cu, Mn, Zn, Ni, Cd, Pb)

Over the course of experiments in bacteria-free systems, Cu concentrations increased by factors of 2.9 and 2.5 for organic and mineral soil horizons, respectively (Fig. 3.7); the correlation coefficient between  $[Cu]_{aq}$  and  $[DOC]$  was as high as 0.94-0.97. Note that bacteria-free soil suspensions demonstrated highly constant pH in the course of experiment (Fig. 3.2), whereas Cu concentration was systematically increasing in these treatments. It follows that the pH evolution in the course of experiment does not control Cu release from soil particles.

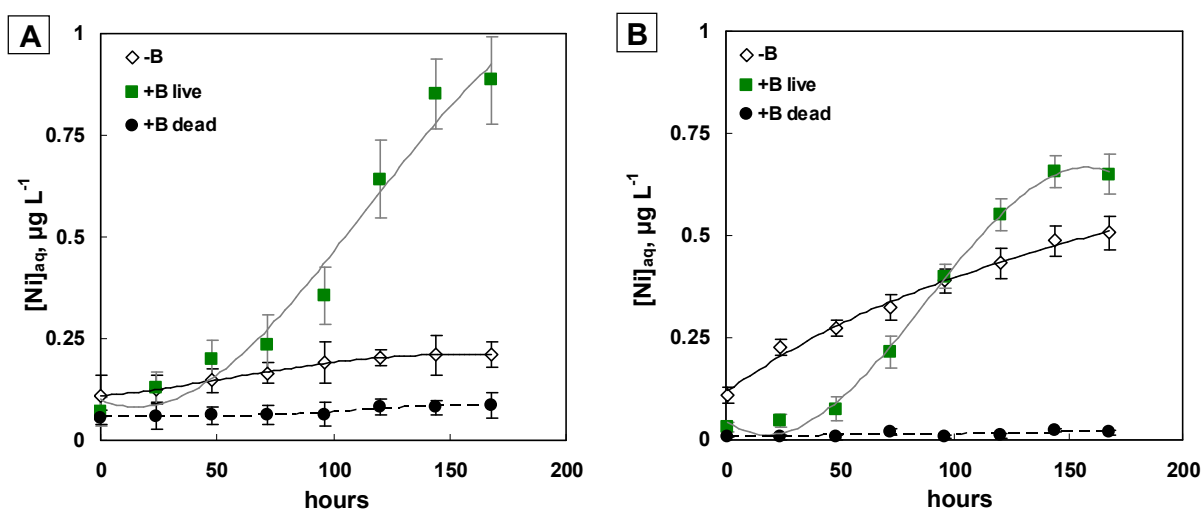


**Fig. 3.7.** Concentration of Cu measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

The presence of live bacteria did not change appreciably the rate constant in either soil horizon and decreased the concentrations relative to bacteria-free treatments in the mineral soil horizon, whereas release ratios were 1.5 to 2 times lower in live-bacteria versus bacteria-free treatments (Table 3.2). We observed quite similar bacterial influences on the concentration

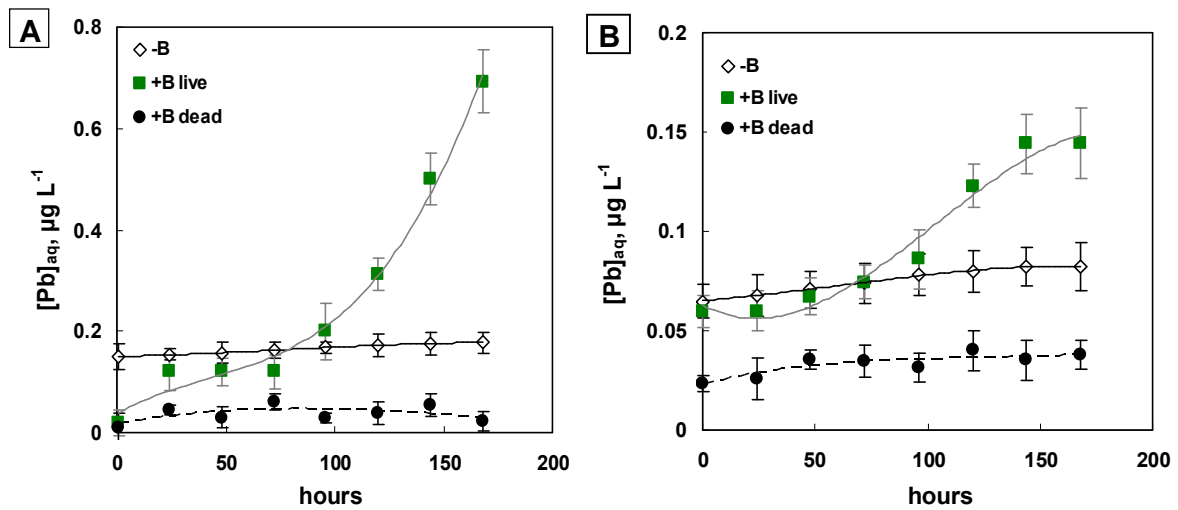
patterns of Mn and Zn in solution: both rate constants and release ratios adhered to the following order: dead bacteria < live bacteria < bacteria-free system (Fig. ESM-6, 7).

The evolution of Ni in experiments yielded values of  $k_{apparent}$  and  $R_R$  that were factors of  $\sim 8$  and  $\sim 3$  higher in live-bacteria compared to bacteria-free experiments for humic and mineral soil horizons, respectively (Fig. 3.8). The behavior of Cd was similar to that of Ni, with the highest concentrations and release rates observed for experiments with live bacteria, in which  $k_{apparent}$  and  $R_R$  values were increased by a factor of  $\sim 4$  for both soil horizons (Fig. ESM-8).



**Fig. 3.8.** Concentration of Ni measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

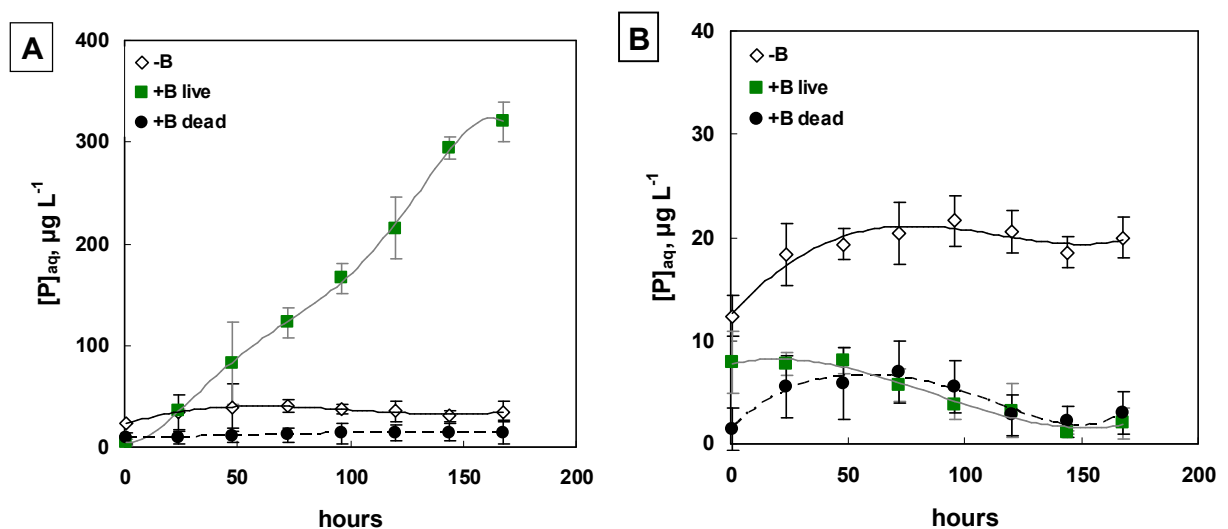
The most pronounced effect of live bacteria was on the Pb release rate, for which the  $k_{apparent}$  is 18 and 6 times higher in live-bacteria compared to bacteria-free experiments for humic and mineral soil horizons, respectively (Table 3.2). The maximal effect of live bacteria on Pb release was observed in the organic-rich soil horizon (Fig. 3.9). It can be seen that, while for dead and bacteria-free system, Pb concentration remains quasi constant for both soils, live bacteria enhance significantly Pb release from soils. Live bacteria increased release ratios (calculated after 100h of experiment) by factors of 34 and 7 for humic and mineral horizons, respectively.



**Fig. 3.9.** Concentration of Pb measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

### 3.3.2.3. Phosphorus and oxyanions

A strong increase in the phosphorus release rate was observed for the humic horizon in the presence of live *P. aureofaciens* relative to abiotic treatment. In contrast, the phosphorus concentration decreased with time in bacterial experiments involving mineral soil (Fig. 3.10). Note that this decrease occurred at P concentrations that were an order of magnitude lower than those obtained in humic horizons.



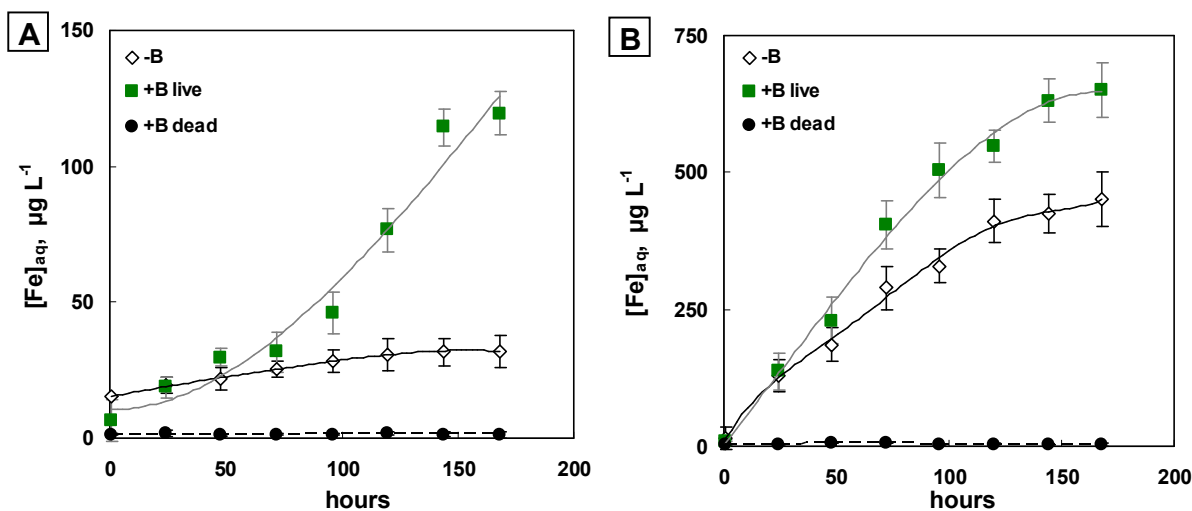
**Fig. 3.10.** Concentration of P measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

The presence of live bacteria enhanced both the release rate constants and release ratio of Mo between aqueous solution and humic soil horizons by factors of 1.5 and 2.0, respectively.

Relative to abiotic experiments, the presence of live bacteria did not modify the rate constant or  $R_R$  of molybdenum in illuvial horizons (Fig. ESM-9, Table 3.2). In contrast to Mo, V release was strongly enhanced in the presence of live bacteria relative to bacteria-free systems, exhibiting increases by factors of 3 and 30 for humic and illuvial soil horizons, respectively (Fig. ESM-10, Table 3.2). Arsenic exhibited rates and concentrations that were 2-times higher in live bacteria relative to bacteria-free experiments in humic soil horizons (Fig. ESM-11A). Meanwhile, mineral soil horizons yielded the lowest As release rates in bacterial experiments (Fig. ESM-11B).

### 3.3.2.4. Trivalent and tetravalent hydrolysates

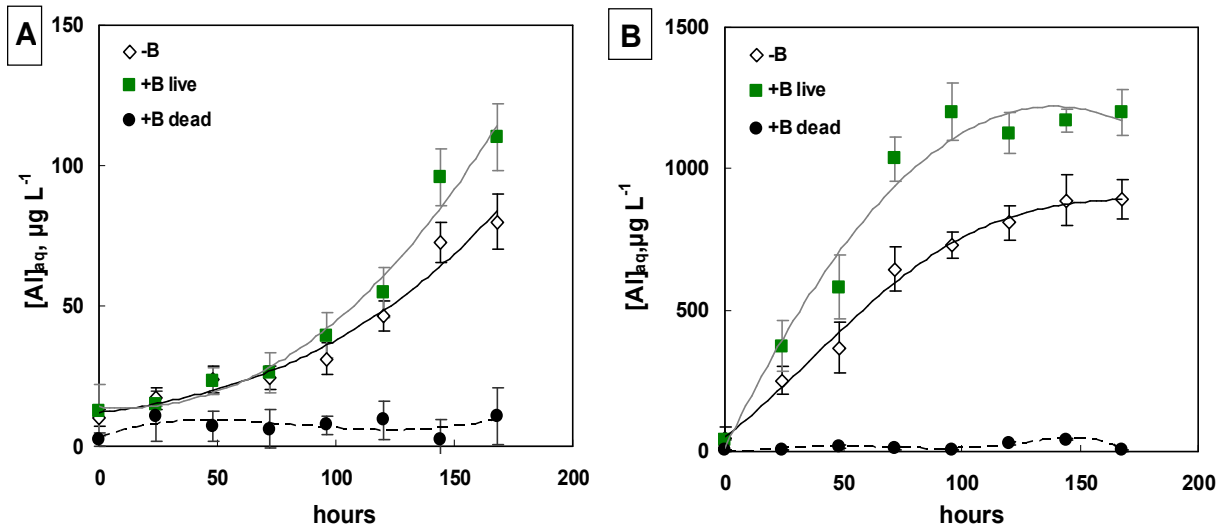
The presence of live bacteria enhanced both the release rate constants and release ratios (between aqueous solution and soil) of Fe by factors of 7 and 1.4 for humic and mineral horizons, respectively (Fig. 3.11).



**Fig. 3.11.** Concentration of Fe measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

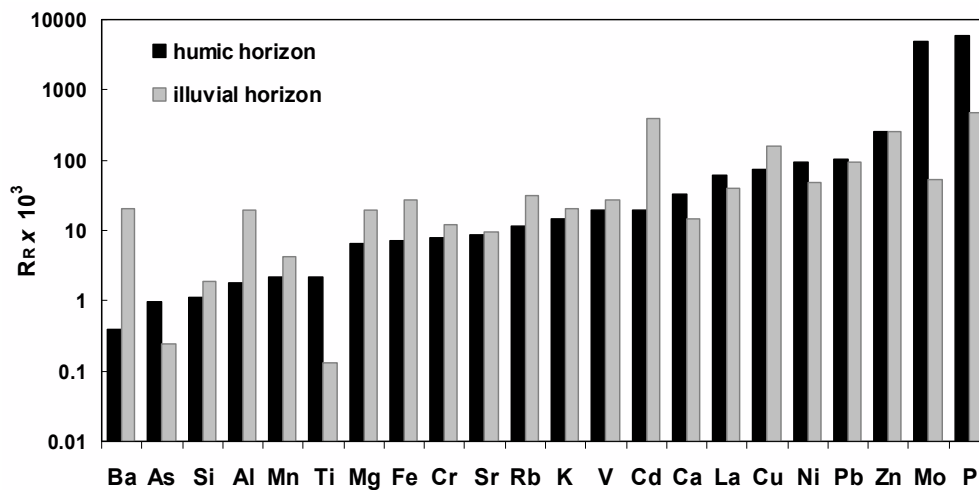
Lanthanum followed the pattern of Fe, demonstrating a significant impact (a factor of ~15) of bacteria in the humic horizon and a much lower effect (a factor of 2.5) in the mineral horizon (Fig. ESM-12). This behavior is in contrast to the behavior of Al, which showed only 30-40% higher release rates and  $R_R$  values in live-bacteria compared to bacteria-free systems for both soil horizons (Fig. 3.12). Live bacteria also had a low impact on Ti, as its concentration

pattern produced the following order of release ratios and apparent rate constants for both soil horizons: dead bacteria < live bacteria < bacteria-free systems (Fig. ESM-13).



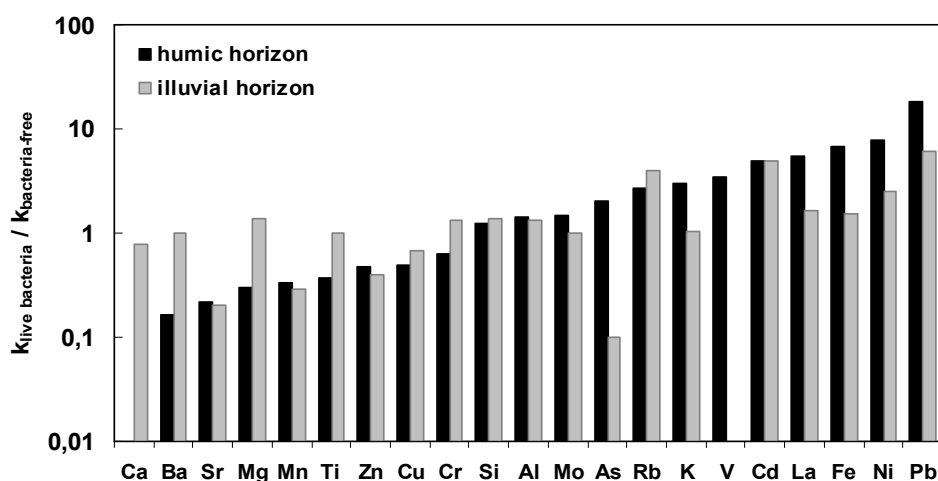
**Fig. 3.12.** Concentration of Al measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 3.2.

The histogram in Fig. 3.13 illustrates the order of the elements' release ratios ( $R_R$ ) between aqueous solution after 160 hrs of reaction and initial soil in the presence of live bacteria. The highest enrichment of solution with respect to both soil horizons was observed for divalent metals Cu, Pb, Ni, Zn, Cd and Mo, whereas Si, Al, Ti, Sr, Ba, As and Mn exhibited the lowest mobility in organic-rich horizons.



**Fig. 3.13.** Apparent release ratio of element between aqueous solution and soil in experiments with live bacteria.

To gauge the degree of bacterial effects on rates of element release, we calculated the ratio of the kinetic constant (Eqn. (1)) obtained in the presence of live bacteria to the kinetic constant obtained in experiments without bacteria (Fig. 3.14). For the humic soil horizon, enhancements observed in the presence of live bacteria increased in the following order: Mo < As < Rb ≤ K < V < Cd < La < Fe < Ni < Pb < P. For the mineral horizon, this order was as follows: Mg ≤ Ni < Rb ≤ Cd < Pb. The inhibiting effect of live bacteria on element release from the humic soil horizon followed the following order: Ca ≤ Ba < Sr < Mg < Mn < Ti < Zn ≤ Cu < Cr < Si ≤ Al. For the mineral horizon, the inhibiting effect of live bacteria was as follows: As < Sr < Mn < Zn < Cu < Ca, whereas the release rates of Ba, Ti, Cr, Si, Al, K, Fe, Mo, P and La were not affected by the presence of live bacteria.



**Fig. 3.14.** The ratio of kinetic constant in the presence of live bacteria to that in bacteria-free experiments.

### 3.4. Discussion

#### 3.4.1. Processes controlling element release during soil interactions with aqueous solutions

Analyses of element group behavior in the course of experiments presented in Section 3.2 followed the basic physico-chemical properties of major and trace elements, their affinity to organic complexes, colloids, and bacterial cells. However there was no consistent concentration evolution within each group, and between organic and mineral soil horizons. Some elements of



the same group exhibited significant response to live bacteria presence and other were indifferent to the presence of bacteria (Figs. 3.13 and 3.14). The element concentration in the fluid phase at time  $t$  reflects the competition between the initial concentration of this element at the beginning of experiment, the release flux of this element from soil ( $F_{\text{release soil}}$ ) and bacterial cells ( $F_{\text{release cells}}$ ), and the removal of this element in the bacterially adsorbed and assimilated forms ( $F_{\text{uptake cells}}$ ) according to:

$$[M]_i = [M]_0 + F_{\text{release soil}} + F_{\text{release cells}} - F_{\text{uptake cells}} \quad (2)$$

However, both the sign and the magnitude of the fluxes change during bacterial growth, following the depletion of the pool of available element in soil, change the physiological requirements of bacteria and cell surface adsorption capacity and modification of the element bioavailability due to the change of its speciation in solution, from allochthonous (humic) complexes at the beginning to autochthonous cell exometabolites and EPS at the end of exposure. The fluxes given in Eq. (2) can be determined in binary systems consisting of filtered ( $< 0.45 \mu\text{m}$ ) cell exometabolites with soils ( $F_{\text{release soil}}$ ) or sterile filtered leachates of soil with live and inactivated cells ( $F_{\text{uptake cells}}$  and  $F_{\text{release cells}}$ , respectively). However, investigating such binary systems was beyond the scope of this study. Below we discuss the effect of pH (and metal speciation), metal complexation/association with DOM and metal adsorption onto cell surface and intracellular assimilation by bacteria as the main factors controlling two counterbalanced fluxes in the system bacteria–soil-DOM-aqueous solution.

#### 3.4.1.1. pH changes during the experiment and various pools of metals in soils

Changes in pH during biotic and abiotic experiments naturally constitute the primary parameter of element dissolution control. The initial pH of all experiments stem from the balance between the initial acidity of soil ( $\text{pH}_{\text{immersion}} = 5.5$  and  $6.0$  for humic and illuvial horizon, respectively, Table 3.1) and the buffering capacity of initial live bacterial suspension ( $\sim 7.0$ , Fig. 3.2). The pH of bacterial suspension without soil decreased by approx. 2 pH units at the end of experiment, reflecting bacterial metabolism as also follows from the DIC production (Fig. ESM-

1). Note that the pH values for different experiments on the same soil horizon are converging at the end of the experiment (see Fig. 3.2), probably demonstrating the drift towards some buffering value of the soil.

The effects of solution pH on element release rates may be linked to 1) chemical speciation of metals in solution, namely the competition between Me-OM complexes and metal hydrolysis, 2) pH-dependence of soil humates' dissolution rates, 3) pH dependence of soil minerals' dissolution rates and 4) TE adsorption on bacterial surfaces and metal desorption from soil particles. These effects can be probed by comparing the dissolution patterns of various cations, for example,  $\text{Al}^{3+}$  versus  $\text{Fe}^{3+}$ . The effect of live *P. aureofaciens* on metal release from humic soil is more pronounced for Fe than for Al (compare Figs. 3.11 A and 3.12 A). At the pH of our experiments with live bacteria (6 to 7), inorganic Al was present in the form of  $\text{Al}(\text{OH})_3^\circ$  and  $\text{Al}(\text{OH})_4^-$  and in the absence of DOM would be strongly supersaturated with respect to  $\text{Al}(\text{OH})_3$  (solid) as follows from MINTEQ calculation (not shown). Despite decreased pH in the presence of live bacteria (cf. Fig. 3.2), which would further decrease Al hydroxide solubility, the Al concentration increased with time, presumably due to complexation with aqueous organic ligands. This is confirmed by vMINTEQ calculations showing that > 99% of Al was present as organic complexes. Note that, while the Al concentration in the mineral horizon is only 20% higher than that in the organic horizon (Table 3.1), Al release from the mineral soil horizon with and without live bacteria is an order of magnitude greater in the former compared to the latter (Fig. 3.12). Because DOC levels were rather similar between the two soil horizons (Fig. 3.3), we hypothesized that 1) the organic pool of Al in soil is much less reactive than the mineral pool of Al or that 2) the most leachable (reactive) fraction of this metal has already been removed from the surface horizon but is still preserved in the deeper (mineral) horizon. The weak effects of live bacteria on Al release rates indicate that bacteria are unable to significantly attack neither the mineral (alumosilicate or Al hydroxide) nor organic (Al humates) pool of Al in soils (Fig. 3.12). While the dissolution rates of Al humates, fulvates, and amorphous organic-rich Al allophones

are virtually unknown, the dissolution rates of a majority of aluminosilicates exhibit weak pH-dependence from  $4.5 < \text{pH} < 7.5$  (Schott et al., 2009), which are the pH conditions under which the experiments herein were performed. As such, the effect of pH change on metal release rates from main soil minerals at the conditions of our experiments is not significant. In contrast, the significant effect of *P. aureofaciens* on Fe mobilization from both humic and mineral horizons may be linked to the metabolism of this bacterium (Emnova et al., 2007). The most likely mechanism of this mobilization is siderophores complexation of  $\text{Fe}^{3+}$ , well known for mobilization of Fe into aqueous solution from Fe oxides (Kraemer, 2004).

When significantly more acidic pH conditions were employed in experiments with dead bacteria ( $< 5$  in the humic horizon and 5-5.5 in the mineral horizon), metal complexation with DOM was prevented compared to biotic and bacteria-free (6 to 7) experiments. This observation may partially explain the significantly smaller release rates of metals observed under these experimental conditions. Above pH 6, the pH itself was unlikely to control element release rates: when the pH was reduced in experiments with live bacteria in the mineral horizon, the concentrations of many elements, including those that did not complex with DOM (such as Rb), increased.

The difference in the initial pH value, metal concentration and status of bacteria created, for some major (Mg, Ca, P, K) and trace (Cu, Pb, Sr, Ba, Mn, Zn, Cd) elements significant offset in the initial element concentration. Although this could not affect the calculated element release parameters (Table 3.2), it hampered straightforward comparison between different treatments. This was resolved via plotting, as a function of time, the M element released fraction instead of its concentration. The fraction is defined as the ratio of  $([\text{M}]_{\text{aq}} - [\text{M}]_0)$  to the initial concentration  $[\text{M}]_0$ , where  $[\text{M}]_{\text{aq}}$  represents element concentration at time  $t$ . Examples of such plots for elements mostly affected by time zero concentration level (Pb, P, DOC, Ba, K, Mn, Zn, Cd and Cu) are shown in Fig. ESM-14. It can be seen that the effect of live bacteria (or the lack of any effect) is systematic, reproducible and statistically significant. Note however that such plots,

while allow to visualize the relative change of element concentration over time, may be partially misleading in the sense that the magnitude of bacterial impact on element concentration cannot be assessed and even large changes in the amount of released element can be strongly underestimated.

#### 3.4.1.2. Metal complexation with DOC, EPS production and heterotrophic consumption of organic complexes.

The fact that DOC concentrations were highest in the presence of live bacteria (compared to the other experimental conditions) could be due to exometabolite production and soluble EPS leaching. This is confirmed by the following order of DOC concentration: live cells > dead cells > soils (Fig. 3.2). Concentrations of DOC were higher in experiments with dead bacteria than in bacteria-free systems, thus suggesting the possibility of cell debris dissolution, cell partial lysis and surface EPS dissolution. Note that significant part of cell debris and EPS was present in colloidal form capable passing through a 0.45  $\mu\text{m}$  filter (e.g., Schijf and Zoll, 2011). In this regard, conventionally filtered TE concentration comprised 4 pools: *i*) inorganic complexes and free metals, *ii*) TE-humic and fulvic complexes and colloids originated from soil OC leaching and *iii*) cell exometabolites and EPS, and *iv*) cell components of < 0.45  $\mu\text{m}$ .

The differences in TE concentrations observed at the beginning of experiments in live-versus dead-bacteria conditions cannot be explained by the presence of EPS on either the cell surface or in the aqueous solution. Although EPS are known to complex with free metal ions (Gadd, 2000; Ledin, 2000; Salehizadeh and Shojaosadati, 2003), the DOC concentration, an indirect measure of EPS, was 3 to 5 times higher for live-bacteria versus bacteria-free experiments (Fig. 3.2), yet initial TE concentrations were often the same. Within the same line of arguments is the observation that the maximum DOC concentration, observed at  $\sim 40$  hrs of exposure for both soil horizons (Fig. 3.2), does not follow the concentration pattern dependence of dissolved divalent metals which are complexed by 90 to 99% with organic matter as confirmed from vMINTEQ calculation ( $\text{Cd}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ), see as an example Figs. 3.2 and

3.9. As such, the bulk DOM does not significantly affect the divalent and monovalent metal release from soil. Rather, the rate of fast-releasing elements ( $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ) is determined by metabolic activity via excretion of strong specific ligands, non-detectable by total ( $< 0.45 \mu\text{m}$ ) DOC analysis and capable of mobilizing these metals from the mineral grains. Note also that the DOC extracts from a humic horizon and from an illuvial horizon of a podzol may have very different properties with regard to metal complexation. Similarly, the properties of DOC may change over the course of the experiment, particularly in the presence of live bacteria. However, quantitative evaluation of the relative importance of these parameters was not possible in this study.

Compared to the other elements studied, the effects of bacteria on P release/concentrations are quite pronounced. Live bacteria greatly increased P release from the humic horizon but clearly inhibited P leaching from the mineral horizon (Fig. 3.10). The forms of dissolved P may be both organic (colloidal) and inorganic (phosphate); however the total ICP MS analysis does not allow to discriminate between them. It is possible that the phosphate released from mineral soil was quickly consumed by live bacteria whereas the organic P from humic horizon was non-bioavailable. Although several bacterial species, including genera *Pseudomonas*, have the ability to solubilize inorganic P compounds (see Gupta et al., 2014 for review), this mechanism could not be confirmed or dismissed in the present work, in which the most important effect of *P. aureofaciens* was observed for organic-rich, upper soil horizons.

Increases in [DIC] with time occurred only in experiments with live cells (Fig. ESM-1) and are most likely linked to on-going bio-degradation of soluble organic products and heterotrophic respiration of soil bacteria (e.g., Bosecker, 1997). The bacterial degradation of soluble humic and fulvic complexes with trace elements may also occur, which would lead to liberation of metals in solution and increases in their concentrations over time (McGrath and Cegarra, 1992). The lack of bacterial effects of Mn, Zn and Cu (Fig. ESM-6, 7 and Fig. 3.7) may be because their associated complexes are too weak for Mn and Zn to be efficiently mobilized

from the soil to the solution. For Cu, on the other hand, the complexes are too strong and, thus, not biodegradable under our experimental conditions. Indifferent behavior of Cu with regard to microbial activity is fairly well known in natural aquatic systems (Luengen et al., 2007; Reynolds and Hamilton-Taylor, 1992; Pokrovsky and Shirokova, 2013). The high correlation coefficient between Cu and DOC concentrations in abiotic experiments suggests that Cu is associated with dissolved organic ligands, facilitating its release from bacteria-free soil into the solution. Simultaneous release of DOC and Cu in solution is reported for sterile sandy loam (Luo et al., 2001). According to vMINTEQ calculation, more than 99% of  $\text{Cu}^{2+}$  is complexed with DOC under experimental conditions of this study.

#### 3.4.1.3. TE adsorption on bacterial surfaces and bacterial intracellular uptake

Compared to bacteria-free systems, the decreases in rates and release ratios of Cu, Mn, and Zn in the presence of live bacteria (Fig. 3.7 and ESM-6, 7) can be explained by their uptake inside the live cells because these elements are potentially biologically active or even limiting with regard to bacterial metabolism. By comparing the rate constants and release ratios obtained after 160 h of reaction (Figs. 3.13 and 3.14), the most significant increases in metal release rates pertain to Ni, Cd, and Pb. These are biologically indifferent or even toxic elements, whose strength of binding to soil components is sufficiently low to explain their low mobilization by bacterial activity. The elevated mobility of these elements in the presence of live bacteria suggests that either 1) these divalent cations exist in exchangeable, easily available (inorganic) complexes or 2) these divalent cations are bound to organic, easily degradable sites. Because these elements are highly mobile in both soil horizons, the second explanation is more likely. In contrast, exchangeable mineral sites might control the behaviors of K, Rb and alkaline-earth metals.

The lack of bacterial effects on Ti, Cr, As and Mo release is also compatible with the formation of weak complexes with anionic species (chromate, arsenate and molybdate) or neutral oxy(hydr)oxide  $\text{Ti}(\text{OH})_4^\circ$ . In contrast, it can be hypothesized that the strengths of complexes

containing  $\text{Ni}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Fe}^{3+}$ , and  $\text{VO}^{2+}$  are optimal for these elements to efficiently facilitate metal extraction from the mineral matrix and subsequent biodegradation in solution by *P. aureofaciens*.

#### 3.4.2. Application to natural environments

Experiments conducted in this study are limited in their direct application to natural environments because the chemical affinity of both abiotic and biotic reactions such as sorption/desorption, coagulation and mobilization of particles, the physiological response of the bacterial cultures and the net rate of reactions may be modified by a different choice of solid solution ratios, stirring, aeration etc. However, via selecting identical experimental conditions for live, dead, bacteria-free and soil-free experiments, one can evaluate the relative role of microbial activity on major and trace element release from soil particles.

From recent kinetics studies of Ca- and Mg-bearing soil minerals in the presence of live bacteria, bacterial exo-metabolites and components of cell envelopes, the following conclusions can be made: *i*) concentrations of 1-10 g/L are necessary to appreciably modify the rates, and *ii*) the effect of live bacteria on the dissolution of basic silicates is virtually absent as a result of the weak impact of bacteria on Si-O-Ca(Mg) bonds (Pokrovsky et al., 2009; Shirokova et al., 2012; Stockman et al., 2012). Live bacteria are only likely to impact the dissolution rate of aluminosilicates via complexation of  $\text{Al}^{3+}$  that has been released into solution, thereby decreasing the concentration of the main rate inhibitor (see Oelkers et al., 1994 and Pokrovsky et al., 2010 for discussion). Therefore, the minimal effects of live *P. aureofaciens* on Si, Ca, and Mg release rates from both mineral and organic horizons observed in the present study (Fig. 3.4, 3.5, 3.6 and Table 3.2) are consistent with available data and surface coordination mechanisms of bacterial impact on mineral dissolution.

The mean concentrations of bacteria are 300-600 and 2000-2500 millions of cells/g soil for taiga and podzol soils, respectively (Aristovskaya, 1965), and the typical bacterial

concentration in European forest soil was reported to be 4800 million cell/cm<sup>3</sup> (Torsvik et al., 2002). Assuming *i*) a typical bacterial cell volume of 1 μm<sup>3</sup>, *ii*) a specific density of cell biomass of ~1 g/cm<sup>3</sup>, and *iv*) a water proportion in soil of 10-30 %, one can calculate that the concentration of bacterial organic matter in soil water is within the range of 1 to 10 g<sub>wet</sub>/L. Because this concentration range is comparable with those in the present study, we can compare the concentrations of major and trace elements in experimental batch reactors to those reported in soil solutions in the field. For this comparison, only the elements exhibiting a steady-state concentration at the end of experiment (pseudo-equilibrium) can be used. A complete survey of TE concentrations in interstitial soil solutions based on dialysis and ultrafiltration techniques is available in the Russian boreal zone of peat and podzol soils (Pokrovsky et al., 2005), which are similar to those studied in the present work.

Al concentrations between 1200 and 2000 μg/L in podzol and peat soil solutions (Pokrovsky et al., 2005) are also comparable to concentrations reported in other podzol soils (Keller and Domergue, 1996; Riise et al., 2000) and are not very different from the concentrations (~1100 μg/L) in the illuvial horizons of the present experiments at which the pseudo-equilibrium was achieved (Fig. 3.12 B). Numerous studies of organic-rich peat and podzol soil solutions from the boreal zone also reported high aluminum concentrations (i.e., 500–2000 μg/L, Strelkova, 1967; Ponomareva and Sotnikova, 1972; Ushakova, 1990; Motuzova and Degtyareva, 1993) and complexation of 90–100% of Al by colloidal organic matter (Nozdrunova, 1965; Kaurichev et al., 1968; van Hees et al., 2001). It is clear that, despite significant binding of Al to OM in both mineral and organic podzol soil layers and the beneficial effect of live bacteria in the present experiments, in which concentrations approach those observed in natural settings, the organic soil layer alone cannot provide naturally relevant concentrations, and the presence of silicate soil minerals from the illuvial horizon is necessary. A similar conclusion is reached for Fe, for which experimental concentrations in mineral soil horizons (400-600 μg/L, Fig. 3.11 B) were similar to those of peat (330-490 μg/L) and podzol



(330  $\mu\text{g/L}$ ) soil solutions (Pokrovsky et al., 2005). La, which is present mostly in the form of Fe-organic complexes, followed the behavior of Fe and exhibited final concentrations in experiments with mineral soil (0.15-0.2  $\mu\text{g/L}$ , Fig. ESM-12) that were similar to those obtained in podzol and peat soil solutions (0.26  $\mu\text{g/L}$  and 0.17-0.37  $\mu\text{g/L}$ , respectively, Pokrovsky et al., 2005). Another example is Ni concentration measured at the ends of live-bacteria experiments in both organic and mineral soils (0.85 and 0.65  $\mu\text{g/L}$ , respectively, Fig. 3.8) that was close to that observed in peat (0.9 to 1.9  $\mu\text{g/L}$ ) and podzol (0.80  $\mu\text{g/L}$ ) soil solutions (Pokrovsky et al., 2005).

In terms of the similarity between the concentrations obtained at the ends of the experiments herein and those encountered in natural interstitial soil solutions, all measured elements can be separated into the following groups: (i) agreement is within 30% for Fe, Al, La, Cr, and Ni; (ii) natural concentrations are 3 to 2 times higher than experimental values for Cd, Pb, Cu, Rb, and Si; (iii) natural concentrations are  $\sim 10$  times higher in podzol soil solutions for Ca, Mg, K, Sr, Ba, Ti, Mo, Zn, As and V and, finally, (iv) Mn concentrations are two orders of magnitude higher in natural soil solutions compared to laboratory fluids. The first two groups of TEs can be considered together, given that 1) natural interstitial soil solutions contain 30 to 60 mg/L of DOC, 2)  $[\text{Me}^{2+}]$  increases linearly with [DOC] in podzol and peat soil solutions (Pokrovsky et al., 2005) and 3) extrapolation of experimental results to naturally relevant [DOC] (40 to 60 mg/L) will increase metal concentrations by a factor of 2. Differences in concentrations of alkaline-earth metals, K, Ti, As and V in the experiments herein compared to those obtained in natural settings can be explained by either 1) the relatively weak sensitivities of these metals to the presence of live bacteria or 2) the different mineral hosts of these elements in boreal soils (Pokrovsky et al., 2005) and subarctic podzol (this study). Finally, the significant difference in Mn concentrations measured in the laboratory versus the field may be due to the different sources of Mn in natural ecosystems versus individual soil mineral and humic samples. In fact, high Mn concentrations in natural soil fluids may originate from downward migration of fresh vegetation and litter leachates, providing significant (100 to 1000  $\mu\text{g/L}$ ) Mn concentration in

upper soil solutions (cf., Viers et al., 2013). This effect may also be observed for Si, K, Rb, Mo and Zn, whose concentrations in plant biomass are much higher than in soil constituents.

In this section, we aimed at first-order comparison of element concentration in interstitial soil solutions with that measured at the steady state conditions in laboratory experiments. It would be premature at this stage to relate the coincidence or not of experimental and natural element concentrations to one well defined factor. Instead, we conclude that batch experiments with live soil bacteria and two soil horizons may serve as adequate laboratory model suitable for examining the complex relationship between element release from soil particles, their complexation with allochthonous and autochthonous DOM and adsorption or assimilation by bacteria. As such, the obtained results may have straightforward application to natural systems allowing *i*) quantifying the effect of the presence of live versus dead soil bacteria on major element release from both organic and mineral soil horizons; *ii*) assessing the maximal possible effect of bacteria on steady-state concentration of trace metals in soil suspension and *iii*) constraining two possible sources of elements in soil solutions: soils (mineral or humic) or organic (plant litter) degradation.

### **3.5. Conclusions**

This work represents an attempt of rigorous comparison of major and trace element release from soil in the presence of bacteria. Thorough interpretation of mechanisms controlling element behavior in ternary system is at present impossible due to *i*) lack of adequate element speciation modeling in the presence of both cell exometabolite and allochthonous organics; note that the TE coprecipitated in the organomineral colloids cannot be properly modeled by available codes (see Vasyukova et al., 2012); *ii*) poor knowledge of chemical status of the element in soil, for which high-resolution synchrotron-based analyses are necessary; *iii*) poor knowledge of thermodynamics and kinetics of element (including even divalent metals) adsorption on the cells surfaces versus intracellular uptake from DOC-rich aqueous solutions. However, distinguishing

several contrasting group of elements with regard to their vulnerability to bacterial presence allow us comparing the experimental steady-state aqueous concentrations with natural cases as a first step towards mechanistic and predictive understanding of major and TE behavior in organic-rich soils of the boreal zone.

The release of major and trace elements from mineral and organic horizons of podzol soil was studied in the presence of live and dead bacteria in batch reactors. Relative to bacteria-free experimental conditions, live bacteria enhanced the release of Fe, V, Rb, Ni, Cd, and Pb from both soil horizons. In contrast, the release of K, Ca, Sr, Ti, Zn and Mn from soil decreased in the presence of bacteria, whereas Cu, Al, Ni, Mo and Cr release from soil was not affected by the presence of bacteria. Among various mechanisms operating during TE leaching from soil to solution, pH changes, element speciation and DOC production and consumption by bacteria, taken individually, do not explain the time – concentration pattern of all TEs. The lack of bacterial influence on the release of major elements (Ca, Mg, Si) from soil minerals is consistent with a very weak effect of bacteria on the hydrolysis of Me–O–Si bonds, as follows from the surface coordination mechanism of silicate dissolution. It is possible that the complex of physico-chemical and biological factors is responsible for the particular behavior of each trace element. The maximal effects of bacteria on divalent metals, such as Ni, Cd and Pb, may stem from the bacterial degradation of organic complexes of these metals in soil and their release into aqueous solution. Presumably, the susceptibility of bulk soil and adsorbed organic complexes to enzymatic degradation is optimal in the case of these metals. The concentrations of trace metals observed at the end of experiments in mineral soil horizons agree reasonably well with concentrations reported in natural soil solutions. Therefore, biotic experiments regarding soil reactivity reasonably approximate the naturally relevant processes involved in soil interaction with fluids in the presence of bacteria.

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## References

- Asmala E, Autio R, Kaartokallio H, Stedmon CA, Thomas DN (2014) Processing of humic-rich riverine dissolved organic matter by estuarine bacteria: effects of predegradation and inorganic nutrients. *Aquat Sci* 76: 451–463.
- Aristovskaya TV (1965) *Microbiology of Podzol Soils*: Nauka, Moscow (in Russian).
- Baranimotlagh M, Gholami M (2013) Time-dependent zinc desorption in some calcareous soils of Iran. *Pedosphere* 23: 185–193.
- Barker WW, Welch SA, Banfield JF (1997) Biogeochemical weathering of silicate minerals. In Banfield JF, Nealson KH (ed) *Geomicrobiology: Interactions Between Microbes and Minerals*. Mineralogical Society of America, *Reviews in Mineralogy* 35, pp. 391–428.
- Benedetti, M., Milne, C., Kinniburgh, D., van Riemsdijk, W., Koopal, L., 1995. Metal ion binding to humic substances: Application of the non ideal competitive adsorption model. *Environ. Sci. Technol.* 29, 446-457.
- Bennett PC, Rogers JA, Hiebert FK, Choi WJ (2001) Silicates, silicate weathering, and microbial ecology. *Geomicrobiol J* 18: 3-19.
- Bosecker K (1997) Bioremediation: metal solubilization by microorganisms. *FEMS Microbiol Rev* 20: 591-604.
- Davis KJ, Nealson KH, Lüttge A (2007) Calcite and dolomite dissolution rates in the context of microbe-mineral surface interaction. *Geobiol* 5: 191–205.
- Emnova E, Tate III RL, Gimenez D, Dascalu A, Toma S, Gojinetschi O, Senicovscaia I (2005) Reduction of metal and water stress of soybean by inoculation with exopolysaccharide producing strains of *Pseudomonas* sp. In: *Natural products: Technologies for their Capitalization in Agriculture, Medicine, and Food Industry. II International Symp Advanced Biological Technologies and their Impact on Economy*, pp. 128-136.
- Emnova EE, Varbanets LD, Vasiliev VN, Ciocarlan AG, Brovarskaia OS, Caunova NJu, Ganea OG, Toma SI (2007) Properties of exopolysaccharides from rhizospheric fluorescent

bacteria of *Pseudomonas* genus. Bulletin of Moldovan Academy of Sciences. Life Sciences. 1 (310): 14-20.

Eusterhues K, Neidhardt J, Hädrich A, Küsel K, Totsche KU (2014) Biodegradation of ferrihydrite-associated organic matter. *Biogeochem* 119: 45-50.

Fein JB, Brady PV, Jain JC, Dorn RI, Lee JU (1999) Bacterial effects on the mobilization of cations from a weathered Pb-contaminated andesite. *Chem Geol.* 158(3-4): 189-202.

Feng M, Ngwenya BT, Wang L, Li W, Olive V, Ellam RE (2011) Bacterial dissolution of fluorapatite as a possible source of elevated dissolved phosphate in the environment. *Geochimica et Cosmochimica Acta*, 75 (19): 5785-5796.

Friis AK, Davis TA, Figueira MM, Paquette J, Mucci A (2003) Influence of *Bacillus subtilis* cell walls and EDTA on calcite dissolution rates and crystal surface features. *Environ Sci Technol* 37: 2376–2382.

Gadd GM (2000) Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Curr Opin Biotechnol* 11: 271-279.

González AG, Shirokova LS, Pokrovsky OS, Emnova EE, Martínez RE, Santana-Casiano JM, González-Dávila M, Pokrovski GS (2010) Adsorption of copper on *Pseudomonas aureofaciens*: Protective role of surface exopolysaccharides. *J Colloid Interface Sci* 350: 305–314.

Gupta DK, Chatterjee S, Datta S, Veer V, Walther C (2014) Role of phosphate fertilizers in heavy metal uptake and detoxification of toxic metals. *Chemosphere* 108: 134–144.

Gustafsson, J. WinHumicV for Win95/98/NT, 1999.  
<http://amov.ce.kth.se/people/gustafjp/winhumicv.htm>. A Windows version of MINTEQA2 website, Gustafsson, J.; <http://www.lwr.kth.se/English/OurSoftware/vminteq/index.htm>.

Hutchens E, Valsami-Jones E, Harouyia N, Chaïrat C, Oelkers EH, Mc'Eldoney S (2006) An experimental investigation of the effect of *Bacillus megaterium* on apatite dissolution. *Geomicrobiol J*, 23: 177-182.

Kaurichev IS, Nozdrunova EM, Evseeva R (1968) Water-soluble aluminium in soils of taiga zone. *Proc Temiryazev Acad Agric Sci (Izvestiya Temiryazevskoi Akad Selkhoz Nauk)* 3: 145–151 (in Russian).

Keller C, Domergue FL (1996) Soluble and particulate transfers of Cu, Cd, Al, Fe and some major elements in gravitational waters of a podzol. *Geoderma* 71: 263–274.

Kraemer SM (2004) Iron oxide dissolution and solubility in the presence of siderophores. *Aquat Sci* 66: 3–18.

Ledin, M (2000) Accumulation of metals by microorganisms — processes and importance for soil systems. *Earth-Sci Reviews* 51: 1-31.

Lee JU, Fein JB (2000) Experimental study of the effects of *Bacillus subtilis* on gibbsite dissolution rates under near-neutral pH and nutrient-poor conditions. *Chem Geol.* 166(3-4): 193-202.

Luengen AC, Raimondi PT, Flegal AR (2007) Contrasting biogeochemistry of six trace metals during the rise and decay of a spring phytoplankton bloom in San Francisco Bay. *Limnol. Oceanogr.* 52: 1112-1130.

Luo YM, Yan WD, Christie P (2001) Soil solution dynamics of Cu and Zn in a Cu- and Zn-polluted soil as influenced by  $\gamma$ -irradiation and Cu-Zn interaction. *Chemosphere* 42: 179-184.

Lüttge A, Conrad PG (2004) Direct observation of microbial inhibition of calcite dissolution. *Appl Environ Microbiol* 70: 1627-1632.

McGrath SP, Cegarra J (1992) Chemical extractability of heavy metals during and after long-term applications of sewage sludge to soil. *J Soil Sci* 43: 313-321.

Milne, C.J., Kinniburgh, D.G., van Riemsdijk, W.H., Tipping, E., 2003. Generic NICA-donnan model parameters for metal-ion binding by humic substances. *Environ. Sci. Technol.* 37 (5), 958-971.

Motuzova GV, Degtyareva GV (1993) Forms of iron compounds in soil solutions and melioration waters in Yachroma lowland as an example. *Russ Soil Sci (Pochvovedenie)* 1: 110-114 (In Russian).

Ngwenya BT (2007) Enhanced adsorption of zinc is associated with aging and lysis of bacterial cells in batch incubations. *Chemosphere* 67: 1982-1992.

Ngwenya BT, Sutherland IW, Kennedy L (2003) Comparison of the acid-base behaviour and metal adsorption characteristics of a gram-negative bacteria with other strains. *Appl Geochem* 18: 527-538.

Nozdrunova EM (1965) Some data on alumina forms in surficial soil waters in soils of forest zone. *Doklady Temiryazev Acad Agric Sci* 115(2): 51-55 (In Russian).

Oelkers EH, Schott J, Devidal JL (1994) The effect of aluminum, pH, and chemical affinity on the rates of aluminosilicate dissolution reactions. *Geochim Cosmochim Acta* 58: 2011-2024.

Pokrovsky OS, Dupré B, Schott J (2005) Fe-Al-organic colloids control the speciation of trace elements in peat soil solutions: results of ultrafiltration and dialysis. *Aquatic Geochem* 11: 241-278.

Pokrovsky OS, Viers J, Emnova EE, Kompantseva EI, Freydier R (2008) Copper isotope fractionation during its interaction with soil and aquatic microorganisms and metal oxy(hydr)oxides: Possible structural control. *Geochim Cosmochim Acta* 72: 1742-1757.

Pokrovsky OS, Shirokova LS, Bénézech P, Schott J, Golubev SV (2009) Effect of organic ligands and heterotrophic bacteria on wollastonite dissolution kinetics. *Amer J Sci* 309: 731-772.

Pokrovsky OS, Shirokova LS, Bénézech P, Schott J, Golubev SV (2010) Reply to comment by R.A. Berner on “Effect of organic ligands and heterotrophic bacteria on wollastonite dissolution kinetics”, *Amer J Sci* 309: 731-772. *Amer J Sci* 310: 425-426. doi: 10.2475/05.2010.05.

Pokrovsky OS, Pokrovski GS, Shirokova LS, Emnova EE, González A, Feurtet-Mazel A (2012a) Chemical and structural status of copper associated with oxygenic and anoxygenic phototrophs and heterotrophs: possible evolutionary consequences. *Geobiology* 10: 130-149.

Pokrovsky OS, Probst A, Levieil E, Liao B (2012b) Interactions between cadmium and lead with acidic soils: Experimental evidence of similar adsorption patterns for a wide range of metal concentrations and the implications of metal migration. *J Hazard Mater* 199-200: 358-366.

Pokrovsky OS, Shirokova LS (2013) Diurnal variations of dissolved and colloidal organic carbon and trace metals in a boreal lake during summer bloom. *Water Research*, 47(2), 922–932.

Ponomareva VV, Sotnikova NS (1972) Processes of migration and accumulation of chemical elements in podzol soils, lysimetric observations. In *Biogeochemical Processes in Podzol Soils*, Moscow, pp. 6–54 (in Russian).

Reynolds GL, Hamilton-Taylor J (1992) The role of planktonic algae in the cycling of Zn and Cu in a productive soft-water lake. *Limnol. Oceanogr.* 37, 1759-1769.

Riise G, Van Hees P, Lundstrom U, Strand LT (2000) Mobility of different size fractions of organic carbon, Al, Fe, Mn and Si in podzols. *Geoderma* 94: 237–247.

Salehizadeh H, Shojaosadati SA (2003) Removal of metal ions from aqueous solution by polysaccharide produced from *Bacillus firmus*. *Water Res* 37: 4231–4235.

Schijf J, Zoll AM (2011) When dissolved is not truly dissolved—The importance of colloids in studies of metal sorption on organic matter. *J. Colloid Interface Sci*, 361(1), 137-147.

Schott J, Pokrovsky OS, Oelkers EH (2009) The link between mineral dissolution/precipitation kinetics and solution chemistry. *Rev Mineral Geochem* 70: 207-258.

Shibata SN, Tanaka T, Yamamoto K (2006) Crystal structure control of the dissolution of rare earth elements in water-mineral interactions. *Geochem. J.* 40, 437-446.

Shirokova LS, Pokrovsky OS, Bénézech P, Gérard E, Ménez B, Alfredsson HA (2012) Experimental study of the effect of heterotrophic bacterium (*Pseudomonas reactans*) on olivine dissolution kinetics in the context of CO<sub>2</sub> storage in basalts. *Geochim Cosmochim Acta* 80: 30-50.

Stepanova VM, Pokrovsky OS, Viers J, Mironycheva-Tokareva NP, Kosykh NP, Vishnyakova EK (2015) Major and trace elements in peat profiles in Western Siberia: impact of the landscape context, latitude and permafrost coverage. *Applied Geochemistry* 53: 53–70.

Stockmann GJ, Shirokova LS, Pokrovsky OS, Bénézeth P, Bover N, Gislason SR, Oelkers EH (2012) Does the presence of heterotrophic bacteria *Pseudomonas reactans* affect basaltic glass dissolution rates? *Chemical Geology* 296-297: 1–18.

Strelkova AA (1967) On the influence of litter on composition of lysimetric solutions from podzol soils under fir and Myrtis forest. In *Proceeding of Karelski Institut of Forest Industry*, pp. 114–118.

Sujith PP, Mourya BS, Krishnamurthi S, Meena RM, Loka Bharathi PA (2014) Mobilization of manganese by basalt associated Mn(II)-oxidizing bacteria from the Indian Ridge System. *Chemosphere* 95: 486–495.

Torsvik V, Øvreas L, Thingstad TF (2002) Prokaryotic diversity—Magnitude, dynamics, and controlling factors. *Science* 296(5570): 1064–1066. doi:10.1126/science.1071698.

Ushakova GI (1990) Role of manure in formation of chemical composition of lysimetric water from fir soil litter. *Russ Soil Sci (Pochvovedenie)* 7: 99–108 (In Russian).

Van Hees P, Lundstrom U, Danielsson R, Nyberg L (2001) Controlling mechanisms of aluminium in soil solution - an evaluation of 180 podzolic forest soils. *Chemosphere* 45(6-7): 1091-1101.

Viers J, Prokushkin AS, Pokrovsky OS, Beaulieu E, Oliva P, Dupré B (2013) Seasonal and spatial variability of elemental concentrations in boreal forest larch foliage of Central Siberia on continuous permafrost. *Biogeochem* 113(1-3): 435- 449.

Wilcke W, Baumler R, Deschauer H, Kaupenjohann M, Zech W (1996) Small scale distribution Al, heavy metals and PAHs in an aggregated Alpine Podzol. *Geoderma* 71(1): 19-30.

Wu SC, Luo YM, Cheung KC, Wong MH (2006) Influence of bacteria on Pb and Zn speciation, mobility and bioavailability in soil: A laboratory study. *Environ Pollution* 144(3): 765-773.

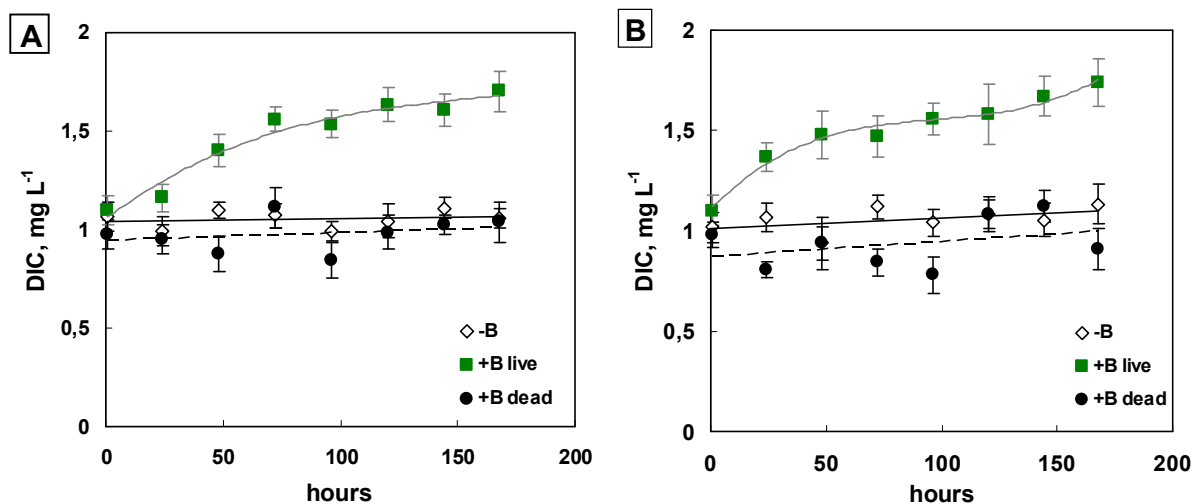
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Wu L, Jacobson AD, Hausner M (2008) Characterization of elemental release during microbe-granite interactions at  $T = 28$ °C. *Geochim Cosmochim Acta* 72(4): 1076–1095, doi:10.1016/j.gca.2007.11.025.

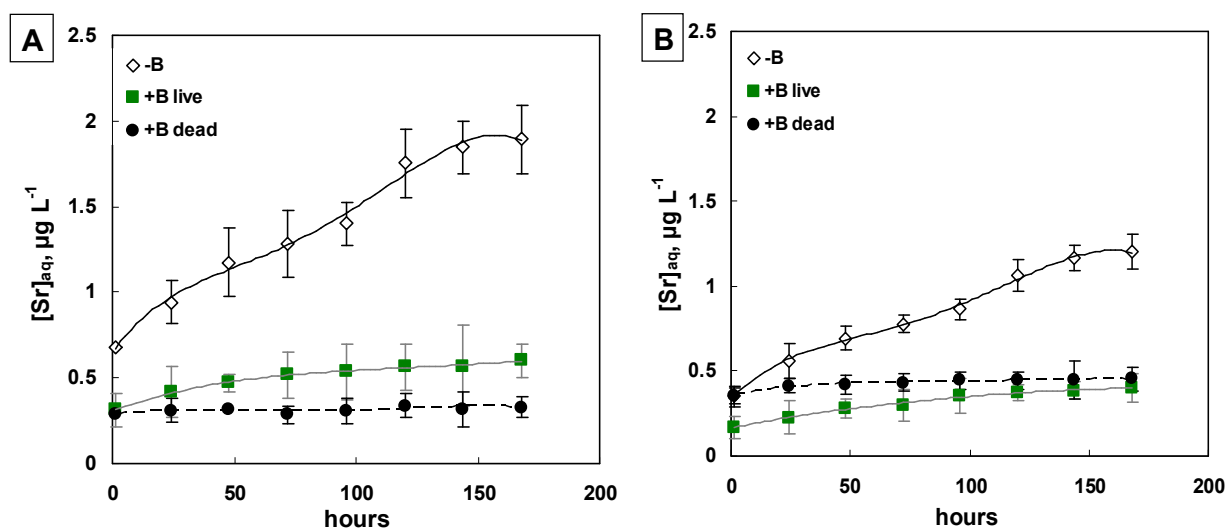


## Electronic Supplementary Material

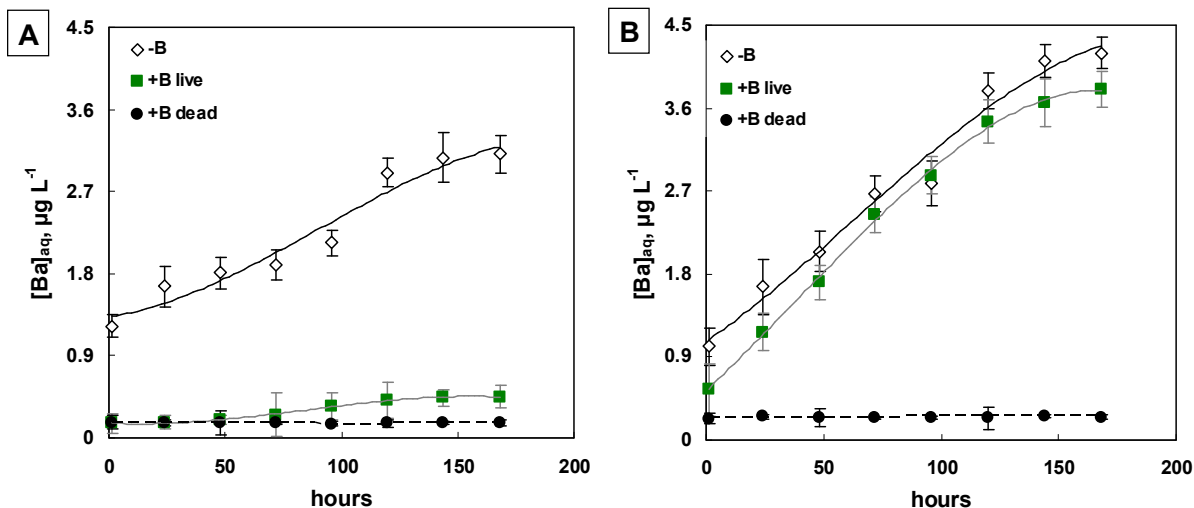
### Experimental evolution of solution component concentration



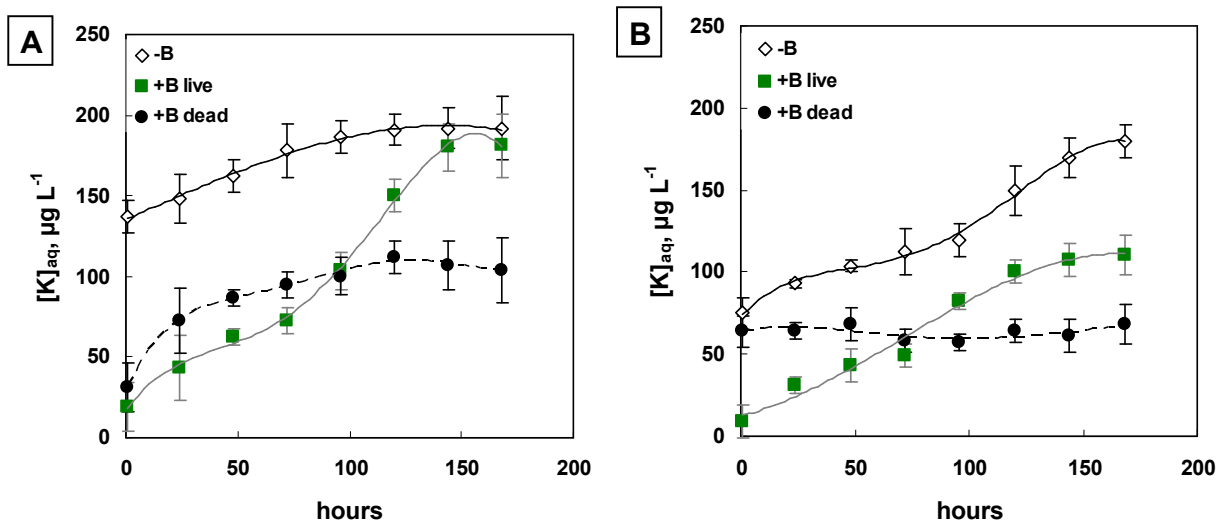
**Fig. ESM-1.** Dissolved Inorganic Carbon measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. 2.



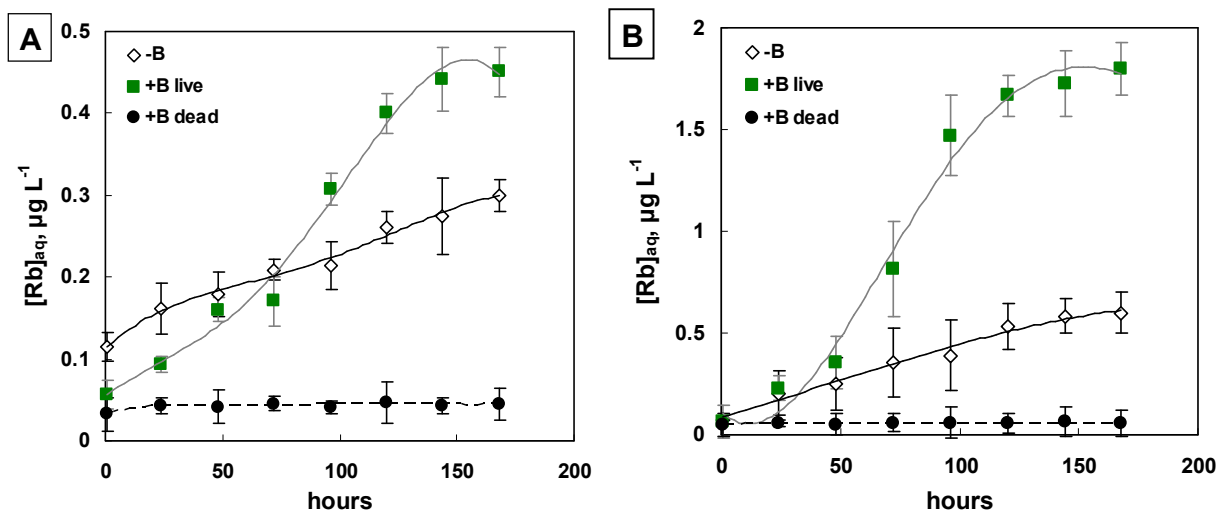
**Fig. ESM-2.** Concentration of Sr measured with humic (A) and illuvial (B) soil horizon. Soil concentration is equal to  $1 \text{ g}_{\text{dry}} \text{ L}^{-1}$ , the biomass of bacteria is  $1 \text{ g}_{\text{wet}} \text{ L}^{-1}$ . The legend abbreviation here and in figures below is as following: open diamonds, -B: bacteria-free system; green squares, +B: live bacteria, and black squares, +B: dead bacteria experiments.



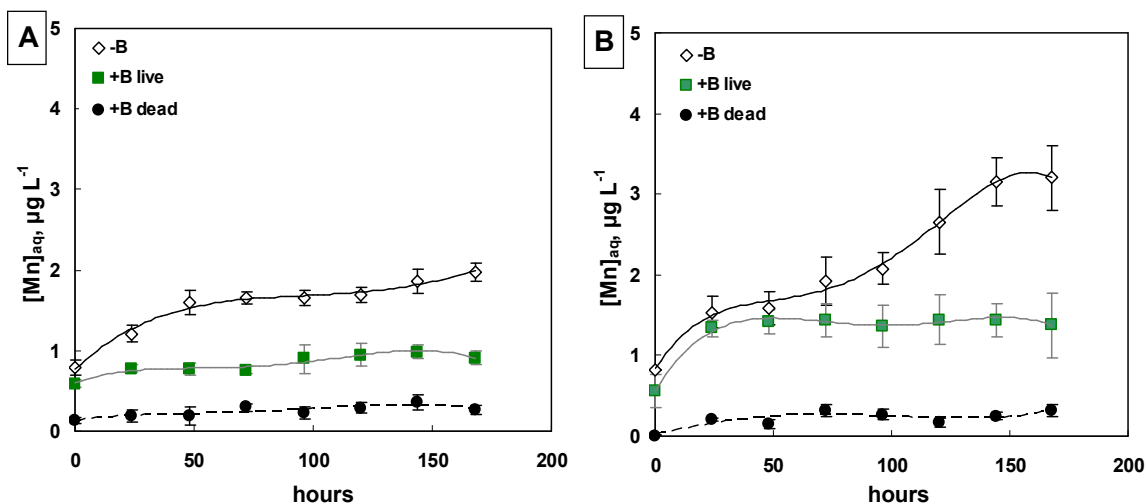
**Fig. ESM-3.** Concentration of Ba measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



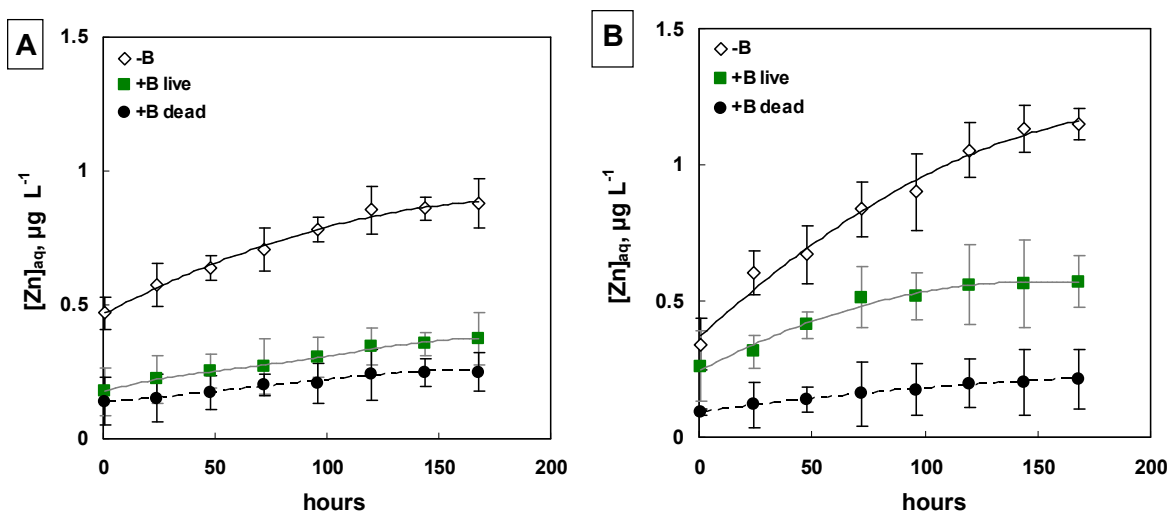
**Fig. ESM-4.** Concentration of K measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



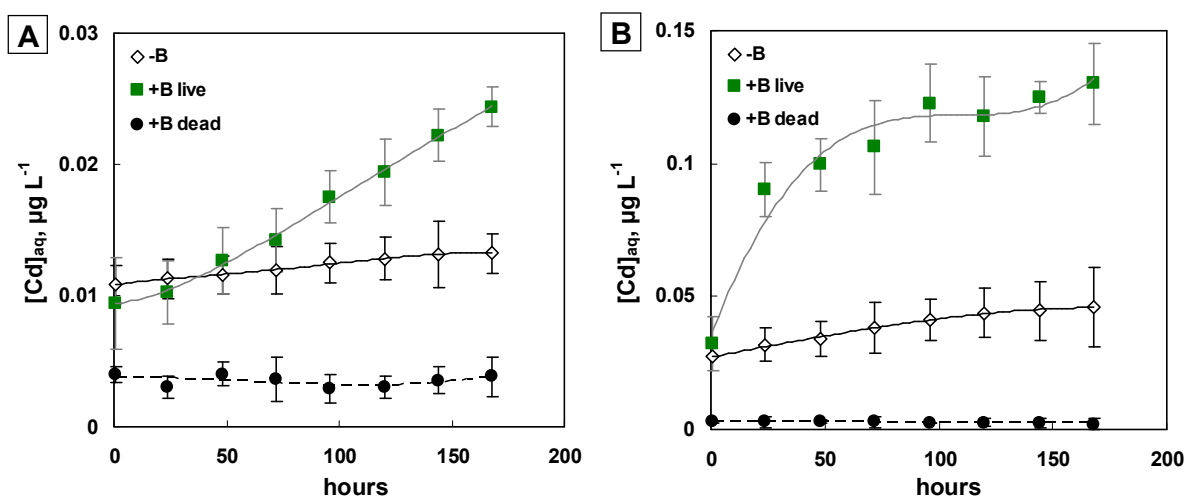
**Fig. ESM-5.** Concentration of Rb measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



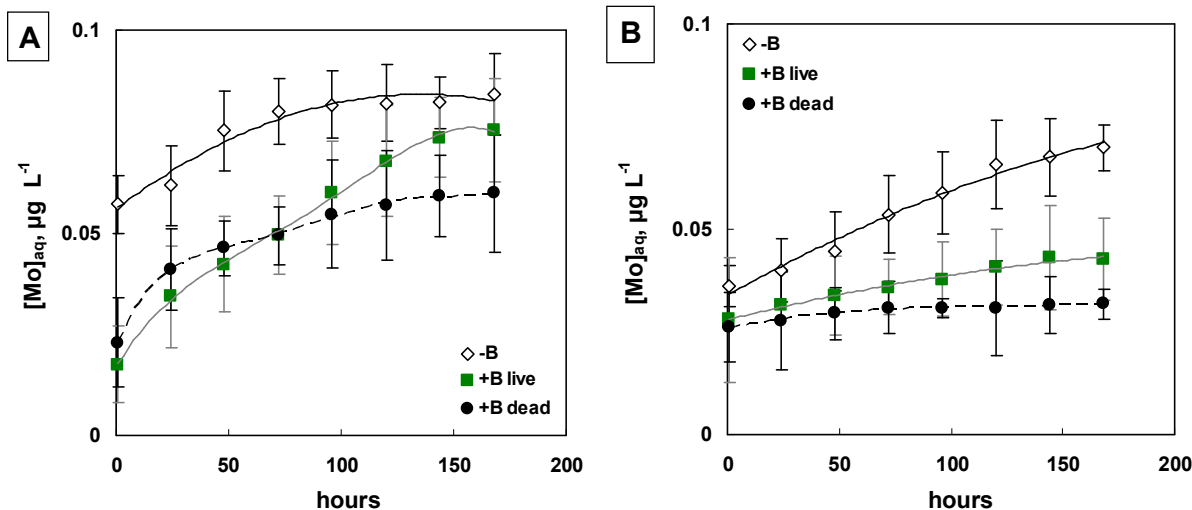
**Fig. ESM-6.** Concentration of Mn measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



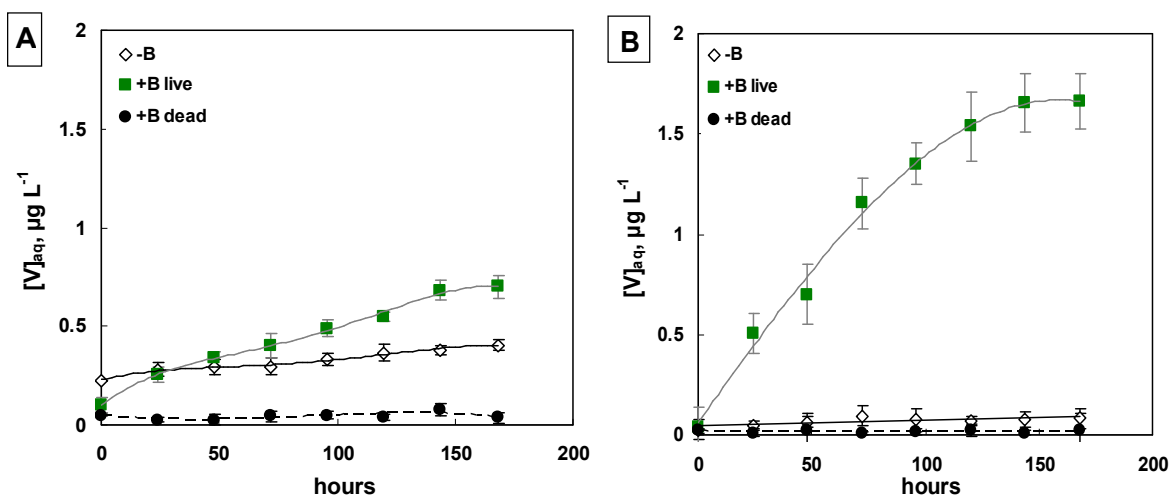
**Fig. ESM-7.** Concentration of Zn measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



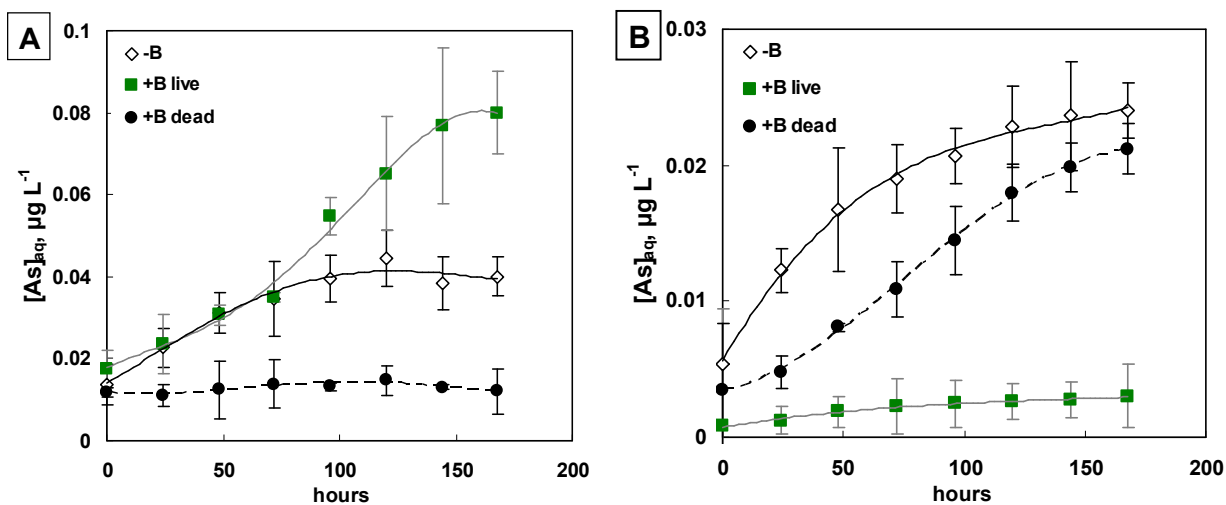
**Fig. ESM-8.** Concentration of Cd measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



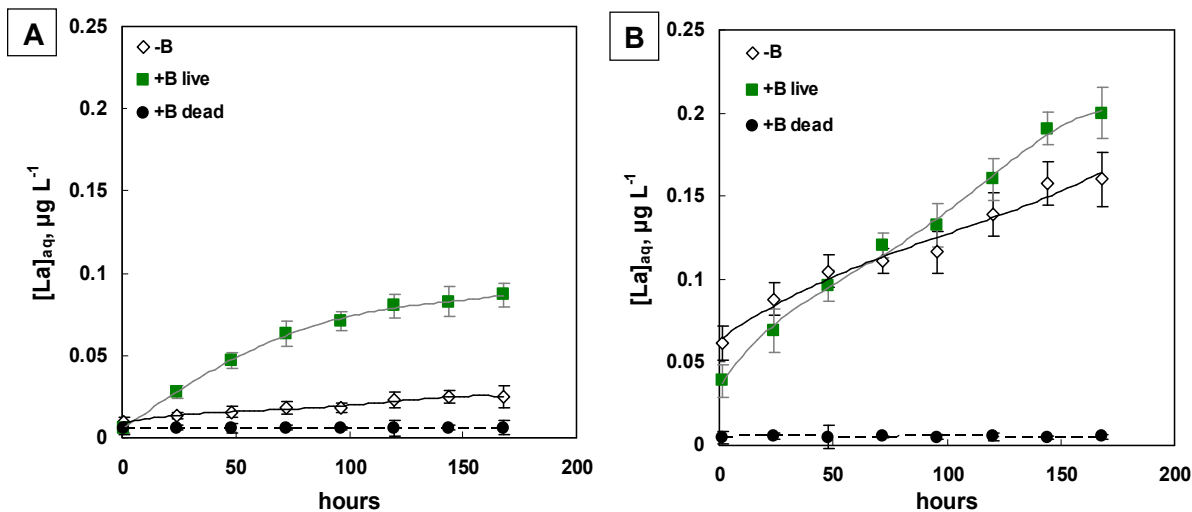
**Fig. ESM-9.** Concentration of Mo measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



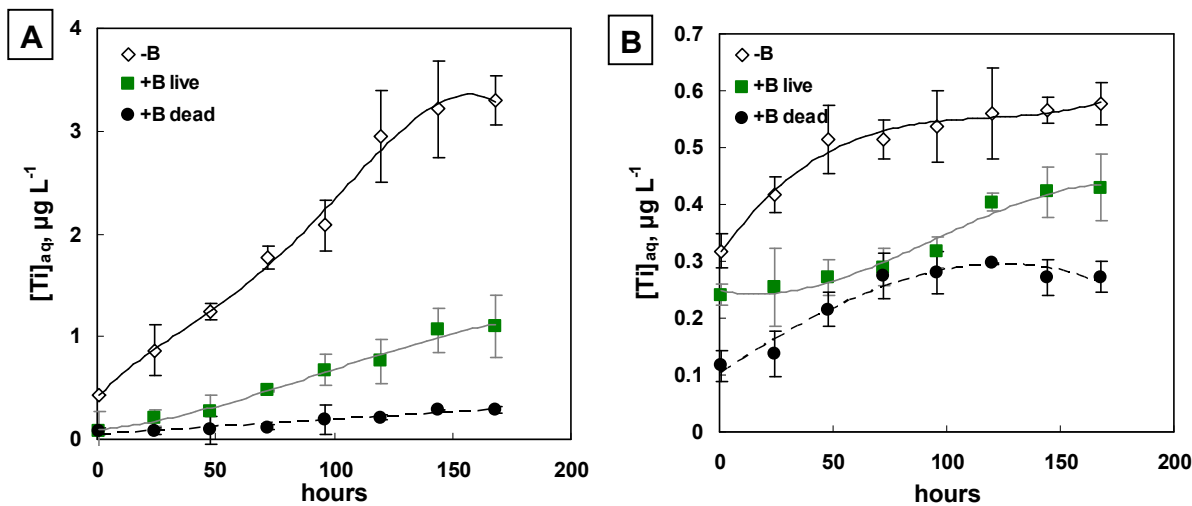
**Fig. ESM-10.** Concentration of V measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



**Fig. ESM-11.** Concentration of As measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-2.



**Fig. ESM-12.** Concentration of La measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-1.

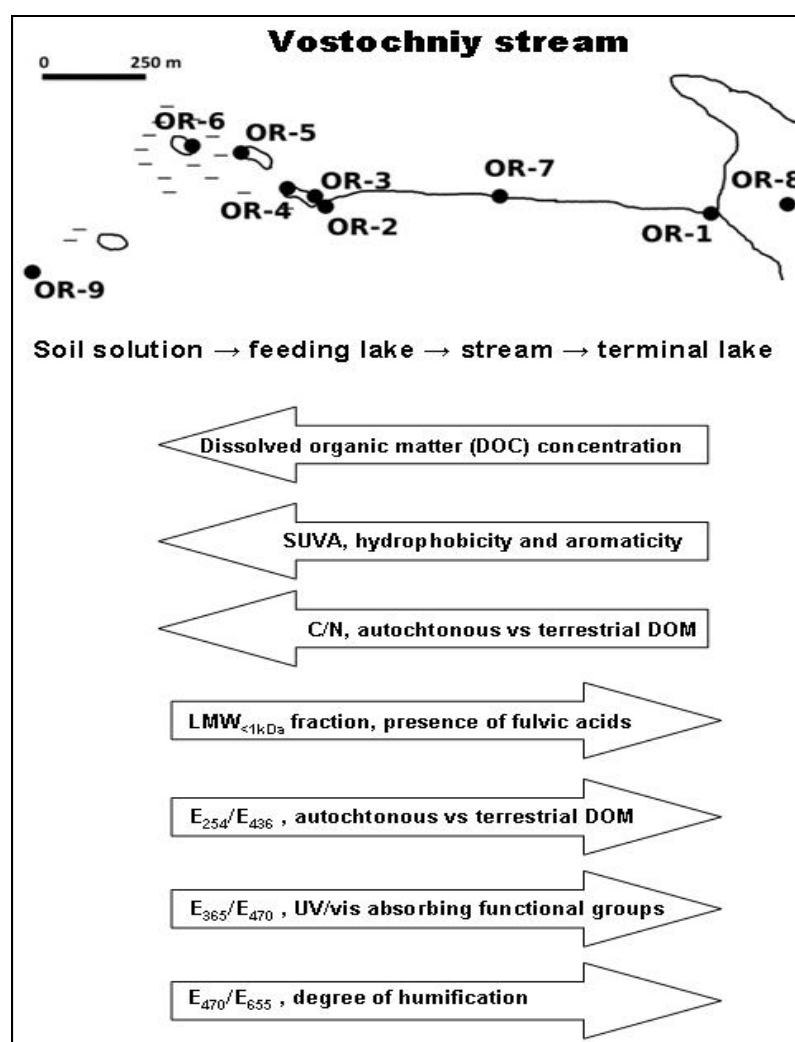


**Fig. ESM-13.** Concentration of Ti measured during experiments with humic (A) and illuvial (B) soil horizons. The experimental conditions and legend abbreviation are the same as in Fig. ESM-1.

# Chapter 4

## Size fractionation and optical properties of dissolved organic matter in the continuum soil solution-bog-river and terminal lake of a boreal watershed

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In general, the soil horizons are source colloids in surface water. In order to better understand the role of colloids in aquatic systems, fractionation methods of environmental samples into particulate, colloidal, and truly dissolved phase are needed. An adequate fractionation procedure involves prefiltration to remove large particles and ultrafiltration to separate colloids from the truly dissolved compounds. In order to study the role of organic colloids in aquatic environments, we used cascade filtration through progressively decreasing pore size.

This work aims to assess the transformation of dissolved organic matter during its transfer from soil to river, within a continuum of soil solution – stream – terminal lake and to assess the main physico-chemical and biological processes responsible for this transformation.

# Size fractionation and optical properties of dissolved organic matter in the continuum soil solution-bog-river and terminal lake of a boreal watershed

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## Abstract

The size distribution and speciation of organic matter (OM) in soil solution, bog, stream, humic and clearwater lake in the north boreal zone (Karelia region, north west Russia) during the summer base-flow period for several years were investigated. The samples were filtered in the field using cascade filtration through progressively decreasing pore size (100, 20, 10, 5, 0.8, 0.4, 0.22, 0.1, 0.046  $\mu\text{m}$ , 100 kDa, 10 kDa and 1 kDa) followed by dissolved organic carbon (DOC) analysis, UV-vis and size exclusion chromatography measurements. Surrogate parameters, such as specific UV absorbance (SUVA; absorbance at 254 nm normalized for DOC concentration in  $1 \text{ mg}^{-1} \text{ m}^{-1}$ ) and the absorbance ratios  $E_{254}/E_{436}$ ,  $E_{280}/E_{350}$ ,  $E_{254}/E_{365}$ ,  $E_{365}/E_{470}$  and  $E_{470}/E_{655}$  (ratio of spectrophotometric absorbance of the sample at two wavelengths) were applied for the characterization of OM in filtered and ultrafiltered water from soil solution, bog, river and lake.

In  $< 0.22 \mu\text{m}$  filtrates, there was a systematic decrease of DOC concentration, C/N ratio, SUVA (hydrophobicity and aromaticity) and proportion of colloidal (1 kDa – 0.22  $\mu\text{m}$ ) OC along the watershed profile from peat bog soil solution, feeding humic lake, to the middle course of the stream towards the terminal oligotrophic lake. Within the filtrates and ultrafiltrates of soil



solution and terminal lake, C/N increases from 100 to 140 and from 7 to 25 for 0.22-10  $\mu\text{m}$  and  $< 1$  kDa fractions, respectively. SUVA, degree of humification, hydrophobicity and aromaticity generally increase from high molecular weight (HMW) to low molecular weight (LMW) fractions, being highest in  $< 1$  kDa fraction. The results allowed a comprehensive view of DOM transport and transformation among various size fractions within a small boreal watershed that can serve as an analogue of small rivers discharge to the Arctic Ocean. It follows that, during the summer baseflow season, the signature of organic-rich interstitial soil solutions originating in a typical peat bog zone can be completely masked by processes occurring in adjacent bog surface waters feeding lakes, as well as in the stream itself. As such, depending on local landscape, one may expect extremely high variability in both chemical nature and MW of DOM delivered by small coastal watersheds to the Arctic Ocean during the summer baseflow period.

**Keywords:** surface waters, dissolved organic matter, boreal zone, UV-vis spectrophotometry, size fractionation, ultrafiltration

## 4.1. Introduction

Over the past decade, there has been a significant rise in interest relating to boreal and subarctic zones, stemming from the governing role of these landscapes in carbon cycle regulation at high latitudes and overall for the planet (IPCC, 2007; Schuur et al., 2008). This is mostly due to (i) a high stock of organic carbon in soils of the northern hemisphere that can be delivered as  $\text{CO}_2$  to the atmosphere as a result of transformation in the aqueous phase and, at the same time, (ii) a significant vulnerability of boreal regions to climate warming. In particular, the increase in dissolved organic carbon (DOC) concentration in surface waters due to climate warming, as observed in the Nordic Countries, the British Isles and the northern and eastern USA (by ca. 10% over 10 yr; Evans et al., 2005; Sarkkola et al., 2009) and on going acidification of boreal surface waters (Reuss et al., 1987; Skjelkvele et al., 2001; Davies et al., 2005; Neal et al., 2008) should inevitably alter both chemical nature of dissolved organic matter (DOM) and its bioavailability.

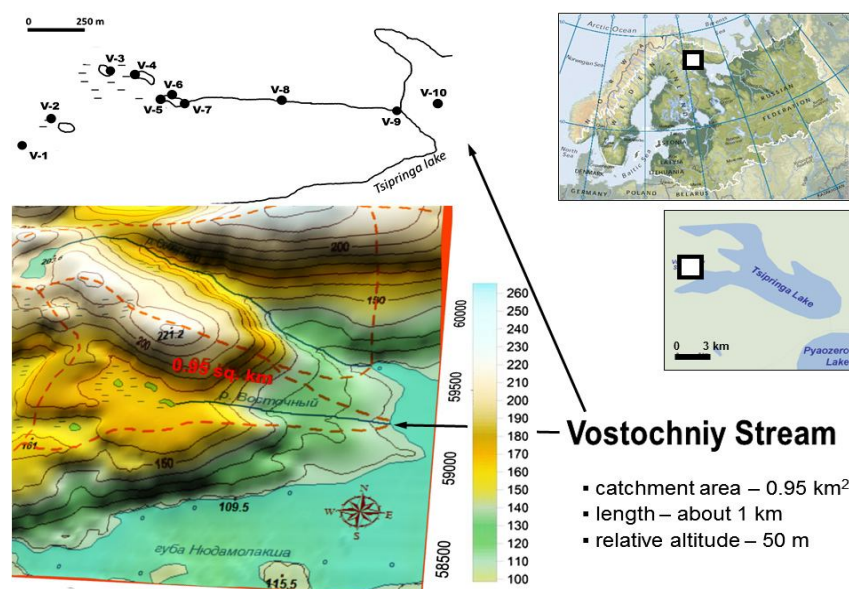
Compared with the significant number of studies devoted to detailed characterization of DOM in lakes (Smith et al., 2004; Hiriart-Baer et al., 2008; Wang et al., 2009; Pokrovsky et al., 2011; Bouillon et al., 2012), rivers (Lara et al., 1998; Callahan et al., 2004; Sachse et al., 2005; Spitzzy and Leenheer, 2007; Huguet et al., 2010; Bourgeois et al., 2011; Selver et al., 2012; Tamooch et al., 2012), mires (Sihombing et al., 1996; Guo et al., 2010; Espinoza et al., 2011; Selberg et al., 2011) and soil solutions (Kalbitz, 2001; Kaiser et al., 2002; Pokrovsky et al., 2005), complete and continuous observations of DOM transformation within a typical landscape continuum in boreal regions are scarce (e.g. Laudon et al., 2011). As a result, prediction of DOM flux and speciation in the terminal reservoirs (Arctic river mouths and large lakes) based solely on the knowledge of soil type and landscape parameters (e.g. % of mire, forest and lake coverage; cf. Bishop and Pettersson, 1996) is difficult if not impossible.

To help gain a better understanding of the main features of the transformation of DOM during its transfer from soil to river, within a continuum of soil solution – stream – terminal lake, we selected a small pristine boreal (subarctic) watershed. The advantage of choosing a small watershed is that it allows testing several hypotheses for the biogeochemical transformation of the DOC in boreal landscape continuum, namely that (i) the change in DOM concentration and its composition (C/N ratio, specific absorbance at 254 nm, light absorbance) from the feedstock soil solution and humic bog lake to the stream and further to the terminal lake should consist of decreasing the C/N ratio and the aromatic (humic) component due to its progressive photo- and biodegradation and its replacement by autochthons organic ligands originating from plankton and peryphyton activity, and (ii) that the relative concentration of soil aromatic (humic) fraction should decrease with the decrease in molecular weight (MW) in a series of filtrates and ultrafiltrates but the decrease may be strongly dependent on the type of sample. It follows that the higher the water residence time in the water body, the higher the degree of allochthonous DOM transformation and thus the terminal (oligotrophic) lake should exhibit the smallest variation in SUVA within the ultrafiltration series compared with small lakes and streams. Finally, we

intended to test the possibility of using the chemical and molecular size parameters of DOM along the watershed profile as an approximation for the nature of the DOM delivered by small coastal organic-rich rivers to the Arctic Ocean. By addressing these hypotheses using frontal cascade filtration/ultrafiltration followed by chemical and spectroscopic analysis we hoped to (i) provide new insight into the mechanisms of DOM migration within the typical small watersheds of the subarctic zone and (ii) allow establishment of the links between the physical and chemical properties and bioavailability of DOM and its possible transformation reactions in the estuarine zone.

## 4.2. Site description

The basin of the Vostochniy stream was chosen for study (Fig. 4.1). It is in the Northern Karelia (N 66°, E 30°), ca. 40–60 km south of the Arctic Circle. The stream flows from west to east and empties into Lake Tsipringa. The lake is ca. 1 km long with a catchment area is 0.95 km<sup>2</sup> and is at a relative altitude of 50 m. The bedrock of the catchment comprises amphibolitic gabbroids of the low Proterozoic intruzive (Ilina et al., 2013b).



**Fig. 4.1.** Sampling sites within the Vostochniy stream watershed.

The climate of the region is mild-cold, transitional between oceanic and continental, with a determinant influence of the Arctic and Northern Atlantic air masses. Average temperature is -

13°C in January and +15°C in July, but extremes can reach -45° to +35°C in the winter and summer periods, respectively. Average annual precipitation ranges between 450 and 550 mm/yr. The snow period lasts from October to April–May, with an average thickness of cover of 70–80 cm. Our study area was in the most elevated part of Karelia, within a landscape of tectonic denudation hills, plateaus and ridges with an average altitude of 300–400 m, with separate insulated massifs (Maksimova, 1967; Vasyukova et al., 2010).

The composition of the river water in Karelia is determined by the weathering of silicate bedrocks of the Baltic crystalline shield and Quaternary deposits, and the presence of numerous peatlands. Typical values for total dissolved solids (TDSs) for the rivers of the region are 15–30 mg/l (Maksimova, 1967; Zakharova et al., 2007) and the concentration of river suspended matter is very low. The soil cover of the region is very young and is often absent from ledges of bedrock and steep slopes. Low temperature, in combination with high humidity, is responsible for the slow humification and mineralization of plant residues. Therefore, much OM has accumulated in the form of peat deposits and, on better drained sites, in the form of coarse humus. Predominant soils are illuvialhumic and illuvial-ferruginous-humic podzols. All the types of podzol exhibit a highly acidic reaction and low base saturation of the upper layers. Coniferous forest (mainly pine and spruce) dominates the vegetation of the region and the common deciduous trees are birch, aspen and alder. The sparse understory consists of mountain ash and juniper, being dominated by blueberries and cranberries in the shrub layer and moss in the lower layer. The rocks are usually covered with patches of black, gray, yellow, red, brown crustose lichens.

### **4.3. Material and methods**

#### *4.3.1. Sampling, filtration*

Fig. 4.1 shows a simplified scheme of the Vostochniy stream watershed sites along with the sampling points, with the list of collected water samples in Table 4.1. The feeding humic

lakes of the bog zone (V-3, V-4, V-5), waterlogged shores of the feeding lake (V-6, V-7), middle course of the stream (V-8), its mouth reach (V-9), interstitial soil solution (V-1) and large clear water terminal lake (V-10) were sampled in the 2008–2013 field seasons during the base flow period. Gravitational soil solution (V-1) of the peat bog zone feeding the watershed was collected from a depth of 5–10 cm with a piezometer. Large volumes (20–30 l) were collected in pre-cleaned, light-protected PVC bottles for the size fractionation procedure, employing 100, 20, 10, 5, 0.8, 0.4, 0.22, 0.1, 0.046, 0.0066 (100 kDa), 0.0031 (10 kDa) and 0.0014  $\mu\text{m}$  (1 kDa) cascade filtration and ultrafiltration conducted directly in the field using a specially prepared polyethylene-covered clean space. The main filtration characteristics are listed in Table ESM-1 (Supplementary material) and the scheme for the size fractionation procedure is given in Fig. ESM-2. The sampling period was always in July, corresponding to summer baseflow. The most complete data series were collected on July 23th in 2009, but some additional series of the Vostochniy stream and adjacent surface streams were performed in 2008, 2009, 2010, 2011 and 2013. The terminal oligotrophic lake (V-10) was sampled in 2008, 2009, 2010, 2011 and 2013. The sampled years were different in mean summer months temperature and precipitation as shown in Table ESM-3.

**Table 4.1.** List of the sampled waters within Vostochniy stream watershed.

Sample	Description	GPS position	
V-1	Soil solution near top feeding lake	N 66°18.489'	E 30°40.707'
V-2	Feeding bog	N 66°18.499'	E 30°40.810'
V-3	Top feeding lake surface ca. 150 m <sup>2</sup> , depth 2.6 m	N 66°18.538'	E 30°40.910'
V-4	Middle feeding lake surface ca. 210 m <sup>2</sup> , depth 3 m	N 66°18.521'	E 30°41.101'
V-5	Low feeding lake, surface ca. 200 m <sup>2</sup> , depth 2.5 m	N 66°18.468'	E 30°41.244'
V-6	Waterlogged shore of another low feeding lake, surface area ca. 50 m <sup>2</sup>	N 66°18.453'	E 30°41.364'
V-7	Waterlogged shore of low feeding lake, surface area ca. 50 m <sup>2</sup>	N 66°18.448'	E 30°41.372'
V-8	Middle course, 600 m from the mouth	N 66°18.460'	E 30°40.973'
V-9	Stream, mouth reach	N 66°18.455'	E 30°42.653'
V-10	Tsipringa lake, 50 m from the mouth reach of the stream	N 66°18.449'	E 30°42.952'

Pre-filtration through 100  $\mu\text{m}$  was performed using a nylon net (Fisherbrand). Cascade frontal filtration with a decreasing pore size from 20 to 0.1  $\mu\text{m}$  was performed using a 250 ml

vacuum polycarbonate cell (Nalgene) and nylon membranes (Osmonics). Frontal cascade ultrafiltration (UF) in the series 100→10→1 kDa was performed using a 400 ml polycarbonate cell (Amicon 8400) equipped with a suspended magnet stirring bar located above the filter to prevent clogging during filtration. Vacuum filtration was performed using a portable hand pump and the ultrafiltration was performed at 2–3 bar using a portable automobile pump with a 0.22  $\mu\text{m}$  Teflon filter installed before the Amicon cell. Large volumes of samples were passed through Laysan (polyethylene terephthalate, PETP) filters of 0.4  $\mu\text{m}$  pore size and 500  $\text{cm}^2$  surface area. Filtration occurred via gravitational flow (0.3–0.5 kPa).

It is known that reproducible and accurate results for size fractionation of DOC require rigorous cleaning and strict sampling protocols (Guo and Santschi, 1996). To this end, before each filtration, the system was cleaned by flushing with EasyPure water, then 3% ultrapure  $\text{HNO}_3$  and, finally, abundant EasyPure water. Each filter was soaked in EasyPure water for at least 1 day before the experiment and used only once. Preliminary experiments demonstrated that flushing 100 ml of MilliQ water (after 1 day's soaking) through the Amicon UF and Nalgen filtration cell with a membrane was sufficient to decrease the OC blank to as low as 0.2–0.5 mg/l, or at least an order of magnitude lower than the typical concentration in filtrates and ultrafiltrates.

During filtration, the first 50 ml of sample solution were discarded, thereby allowing saturation of the filter surface and collecting vessel prior to filtrate recovery. This greatly decreased the probability of cross contamination during sample filtration, while improving the OC blank. It also provided identical conditions of filtration for all samples and allowed good recovery of colloidal particles. Discussion of the technique and precautions against possible filtration artifacts is given by Viers et al. (1997), Dupré et al. (1999), Pokrovsky and Schott (2002), Pokrovsky et al. (2005, 2006, 2010), Pokrovsky and Shirokova, 2013, Alekhin et al. (2010) and Ilina et al. (2013a).

#### 4.3.2. Analytical techniques

Water temperature, pH and conductivity were measured in the field. The pH was measured with an uncertainty of 0.02 using a combination glass electrode calibrated against NIST buffer solutions. Major anion concentrations ( $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ) were measured using ion chromatography (Dionex 2000i) with an uncertainty of 2%. Alkalinity was measured in situ via Gran titration with HCl using phenolphthalein as indicator. DOC concentration was determined in Toulouse using a Shimadzu CNS Analyzer and in Moscow using an Elementar TOC analyzer with an uncertainty of 3% and detection limit of 0.05 mg/l.

Absorption in the range 375–655 nm was measured directly in the field in 0.22  $\mu\text{m}$ -filtered samples using a spectrophotometer (Expert-003) with a 30 mm glass cell equipped with several cartridges having working wavelength of 375, 400, 430, 470, 505, 525, 572, 590 and 655 nm. Absorption spectra of the filtrates over a range of 200–700 nm with 1 nm resolution were also measured in the laboratory within 1 month of sampling, using a Specord 50 instrument.

Specific UV absorbance ( $\text{SUVA}$ ,  $\text{l mg}^{-1} \text{ m}^{-1}$ ) is absorbance of a given sample at 254 nm divided by the DOC concentration of the sample. The ratio describes the nature of the DOM in terms of hydrophobicity and hydrophilicity; a value  $> 4$  indicates mainly hydrophobic and especially aromatic material, whilst a value  $< 3$  corresponds to the presence of mainly hydrophilic material (Edzwald and Tobiasson, 1999; Minor and Stephens, 2008; Matilainen et al., 2011). Several studies have emphasised that good agreement may exist between the ability for OM removal by coagulation and a high  $\text{SUVA}$  value (Archer and Singer, 2006; Bose and Reckhow, 2007).

Weight average MW ( $\text{WAMW}$ ) was measured via size exclusion chromatography (SEC; Hagel, 2001) using an Agilent 1100 chromatographic system (Agilent Technologies, USA) with diode array detector and Ultropac column at 280 nm (TSK G2000SW;  $7.5 \times 300$  mm; LKB, Sweden). A solution of 0.1 M  $\text{Na}_2\text{HPO}_4$  buffer (pH 7) and 0.1% sodium dodecyl sulfate was used as effluent. All samples were purified from low MW (LMW) contaminants by elution

through a Sephadex G-10 column. Calibration was performed with certified globular protein solutions (Pharmacia Fine Chemicals, Sweden).

## 4.4. Results and discussion

### 4.4.1. General hydrochemical parameters

DOC, N and major element concentrations and C/N ratio, WAMW, alkalinity, pH and conductivity values are reported in Table 4.2. The samples from the stream and the terminal Tsipringa Lake were neutral, with pH ranging from 6.3 to 7.5, whereas the water from the waterlogged humic lake and soil solution was slightly acidic, with a pH of 5.8 and 3.6, respectively.

**Table 4.2.** Composition and DOM parameters for 0.22  $\mu\text{m}$  fractions of surface waters (nd, not determined).

sample	V-9	V-7	V-6	V-5	V-4	V-3	V-8	V-10	V-1
pH	6.7	6.3	6.6	5.8	6.6	6.3	6.6	7.5	3.6
T, °C	12.8	18.9	18.3	19.9	20.1	21.7	15.7	17.8	17.2
O <sub>2</sub> , mg/l	4.3	nd	4.2	nd	nd	4.1	nd	4.8	1.9
R <sup>a</sup> , $\mu\text{Sm}/\text{cm}$	14.4	18.1	16.6	15.7	19.1	20.8	14.3	42.5	57.4
TDS <sup>b</sup> , mg/l	8.5	8.2	7.5	8.1	9.7	10.2	7.9	23.3	31.9
Na <sup>+</sup>	0.96	0.75	0.72	0.84	0.88	0.98	0.88	1.2	1.23
Mg <sup>2+</sup>	0.56	0.51	0.55	0.55	0.61	0.59	0.58	1.6	0.49
K <sup>+</sup>	0.04	0.01	0.07	0.04	0.04	0.04	0.06	0.80	0.38
Ca <sup>2+</sup>	2.2	2.3	2.5	2.0	2.5	3.3	2.0	5.9	1.3
SO <sub>4</sub> <sup>2-</sup>	0.89	1.1	0.29	0.97	1.15	0.32	0.84	0.04	0.06
NO <sub>3</sub> <sup>-</sup>	0.39	0.50	0.14	0.61	0.64	0.03	0.64	0.08	0.12
Cl <sup>-</sup>	0.42	0.40	0.38	0.28	0.35	0.33	0.24	0.64	0.69
HCO <sub>3</sub> <sup>-</sup>	17.4	9.2	9.7	9.0	11.0	13.4	9.5	33.1	Nd
( $\Sigma^+ - \Sigma^-$ )/ $\Sigma^+$ , %	-3.6	-3.3	5.2	-3.2	3.3	3.8	-3.1	1.3	65
N, mg/l	0.34	nd	nd	Nd	nd	0.33	nd	0.18	0.49
DOC, mg/l	16.0	18.0	19.0	19.0	18.5	18.0	16.5	7.0	144
C/N	48	nd	nd	nd	nd	58	nd	24	104
WAMW <sup>c</sup>	1010	nd	nd	nd	nd	1020	nd	960	1260
SUVA	4.1	3.2	nd	nd	nd	4.2	nd	1.1	4.9
E <sub>365/465</sub>	4.1	3.7	3.7	nd	nd	3.8	3.9	5.0	2.7
E <sub>465/665</sub>	10.0	5.7	7.1	nd	nd	8.0	10.5	12.0	5.7

<sup>a</sup> Specific conductivity; <sup>b</sup> Total dissolved solid; <sup>c</sup> weight-average molecular weight.

All the samples were low in dissolved solids (TDSs 6–30 mg/l) with a dominance of Ca<sup>2+</sup> and HCO<sub>3</sub><sup>-</sup> in lakes and stream or Ca<sup>2+</sup>, Cl<sup>-</sup> and Na<sup>+</sup> in the soil solution. The inorganic ion charge balance ( $(\Sigma^+ - \Sigma^-)/\Sigma^+$ ) was < 0.1 for all samples except the soil solution, with a deficit of anions of



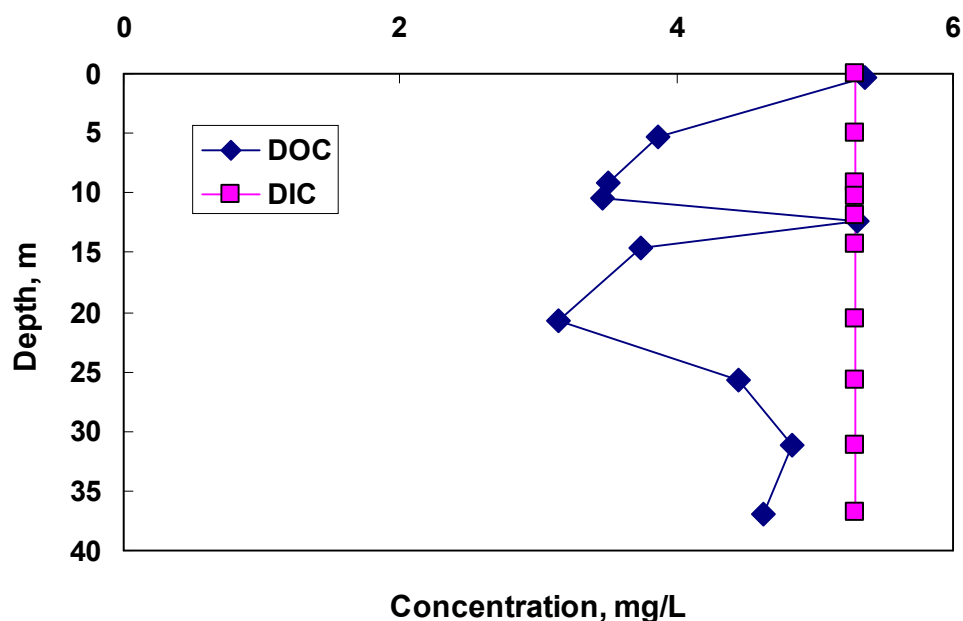
0.4–0.5. The deficit can be explained by a high concentration of DOC (75 mg/l), similar to that reported for other surface waters of North Karelia (Vasyukova et al., 2010, 2012).

#### *4.4.2. Representativity of the samples*

Although our upland sampling locations represent a small, almost negligible, fraction of the Tsipringa catchment, the Vostochniy stream is typical of small streams feeding the lake. This was confirmed by analysis of DOC concentration in the majority of small surface streams reaching Lake Tsipringa and fed by adjacent bog zone and forest areas. Very similar landscape context was observed in the neighboring Palojoki river watershed investigated in our previous studies (Iilina et al., 2013a, b). The mean DOC concentration of 20 small streams representing almost 80% of all surface water feeding of the Tsipringa lake was  $10.5 \pm 6.3$  mg/l. The mean DOC concentration of another 12 surface streams feeding the adjacent Pyaozero lake sampled in July 2009 was  $16.3 \pm 3.2$  mg/l, whereas the mean concentration of 8 surface streams of the region in July 2008 was  $13.1 \pm 4.0$  mg/l (Shirokova et al., unpublished results). These values were very close to the typical value of 16 mg/l in the Vostochniy stream (samples V-8, V-9).

The large, oligotrophic Lake Tsipringa has been studied over several summer sampling campaigns (2008, 2009, 2010, 2011, 2013), yielding an average DOC concentration in the surface water of  $5.55 \pm 1.50$  mg/l. A depth profile of dissolved organic and inorganic carbon obtained in July 2008 is shown in Fig. 4.2. It can be seen that the average DOC concentration of  $5.5 \pm 1.5$  mg/l in the surface layer was within the variation observed in the water column during the summer period. In contrast, highly constant dissolved inorganic carbon concentration suggested an absence of strong underground input, benthic respiration or mineralization of organic detritus in the bottom layer and reflected overall high mixing of the water column and the absence of hypolimnion within the first 30 m, as also followed from our in situ O<sub>2</sub> measurements (not shown). Finally, the representivity of the soil water sample (V-1) was confirmed by high DOC concentration in the soil water systematically observed during several

summer seasons (from 105 to 150 mg/l). It is also noteworthy that, while the soil water was obtained from a peat bog, the Tsipringa catchment is to a large degree forested, suggesting that much of the DOM in the catchment may potentially have a forest origin. However, our previous results for Northern Karelia region (Zakharova et al., 2007) unequivocally prove the dominant role of wetlands (bogs), not forests, in supplying the DOC in river catchments of this boreal zone.



**Fig. 4.2.** Dissolved organic and inorganic carbon (DOC and DIC, respectively) concentration as a function of depth in the Tsipringa Lake (V-10) sampled in July 2009.

Given the size of the water bodies and the debit of the surface water flow measured at point V-8 of the Vostochniy stream in July 2009, during a normal year of atmospheric precipitation, the water residence time in the water bodies could be ranked as follows: interstitial soil solution (V-1) < waterlogged shores of the feeding lake (V-6, V-7) ≤ Vostochniy stream itself (V-8, V-9) << feeding humic lakes of the bog zone (V-3 < V-4 < V-5) << large clear water terminal Lake Tsipringa (V-10). Within this series of water bodies, the water residence time ranged from hours (V-1, V-6, V-8, V-9) to days/weeks (V-3, V-5) and months (V-10).

#### 4.4.3. Spatial variation in OC characteristics

##### 4.4.3.1. DOC concentration

The variation in DOC concentration in several filtrates along the landscape profile of the stream is plotted in Fig. 4.3. There was a systematic DOC decrease down the catchment, from soil solution (V-1) through the feeding bog (V-3) and small feeding lakes (V-4, V-5, V-6, V-7), along the stream itself (V-8, V-9) and finally to the terminal Tsipringa lake (V-10). The largest decrease occurred between soil solution (V-1) and bog lake (V-3) and between the mouth of the stream and the terminal clear water lake; the variation within the upper humic lakes and within the stream was rather small (< 10%). This pattern was highly reproducible from one year to another although the absolute values of DOC concentration were different, depending on the precipitation level and stream discharge.

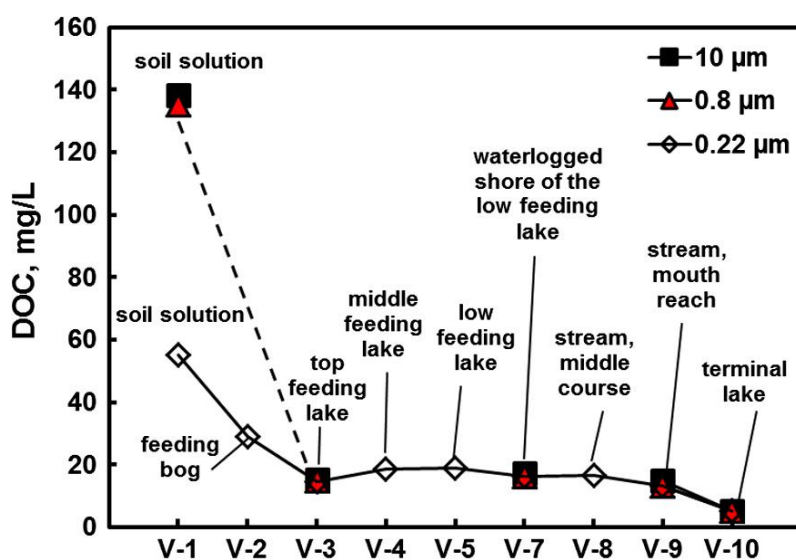


Fig. 4.3. DOC concentrations (mg/l) for samples of Vostochniy stream.

##### 4.4.3.2. C/N ratio

The concentration of dissolved organic N (DON) and C/N decreased systematically along the watershed profile, from soil solution through stream and to the terminal lake (Table 4.2), and the higher the DOC concentration, the higher C/N. The C/N value for the soil solution was 104, similar to the biomass of coniferous trees (Onstad et al., 2000; Twichella et al., 2002; Tremblay and Benner, 2006), confirming the dominant role of the lignocellulose complex of pine and birch

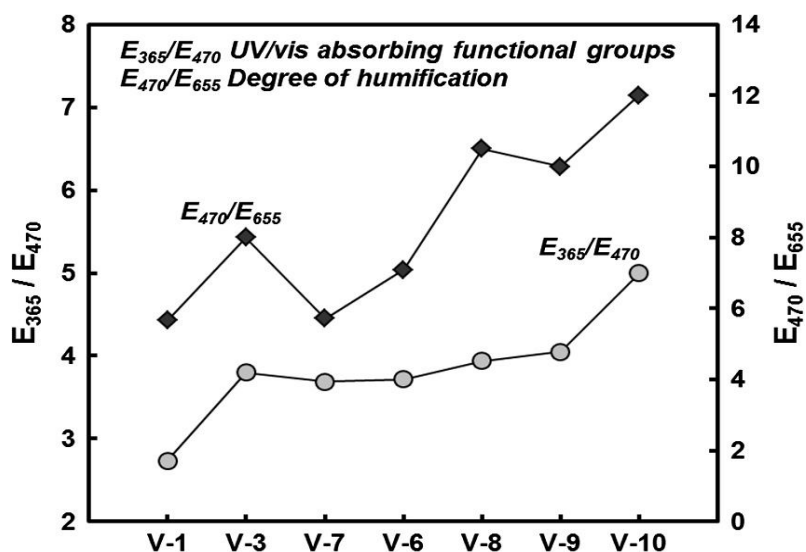
litter in the formation of the aqueous OM of peat bog soil water (Guggenberger et al., 1994; See and Bronk, 2005; Tremblay and Benner, 2006).

The samples from the upper lakes (V-3 to V-6) and the stream (V-7 to V-9) were very similar to each other but drastically different from the bog soil water. As such, the dominant source of OM in the stream should be bog lakes rather than interstitial peat soil solution. The water of terminal Lake Tsipringa had the lowest C/N value (24), which is typical for aquatic phytoplankton and macrophytes and their humification products (Wolfe et al., 2002). Therefore, the contribution from allochthonous river water and bog water to the DOM pool of this large oligotrophic lake was rather small.

#### 4.4.3.3. *Optical characteristics*

The SUVA values for the water samples from the Vostochniy watershed profile ranged from 1.1 to 4.9 l mg<sup>-1</sup> m<sup>-1</sup>, being maximal for the soil solution and minimal for the oligotrophic lake (Table 4.2). The E<sub>365</sub>/E<sub>470</sub> ratio is used for characterizing the functional group absorbances in the UV and visible range (Uyguner and Bekbolet, 2005). E<sub>365</sub>/E<sub>470</sub> values along the watershed profile systematically increased from soil solution towards the terminal lake (Fig. 4.4). A similar trend occurred for E<sub>470</sub>/E<sub>655</sub>, which increased by a factor of two from aqueous extract of soil litter to the lake (Fig. 4.4). This ratio is known to correlate with the degree of condensation of DOM aromatic groups, or the degree of humification (Chin et al., 1994; Stevenson, 1994; Hur et al., 2006). The lowest value was encountered for the soil solution and waterlogged bog lakes feeding the stream (V-6 and V-7). The values are similar to those reported for soil humic acids (Schnitzer and Calderoni, 1985; Adani et al., 2006). For the other samples, E<sub>470</sub>/E<sub>655</sub> was significantly higher, which may be linked to the presence of a high concentration of LMW fulvic acids (Fig. 4.4), similar to results from Chen et al. (1977) and Uyguner and Bekbolet (2005). In accord with the data for DOC, both E<sub>365</sub>/E<sub>470</sub> and E<sub>470</sub>/E<sub>655</sub> parameters increased from soil solution towards the terminal lake during several years of observation (2009,

2010 and 2013), although the absolute values were rather variable, depending on the degree of atmospheric dilution of the soil, bog and river water.



**Fig 4.4.**  $E_{365}/E_{470}$ ,  $E_{470}/E_{655}$  ratios for the samples (V-1 – soil solution, V-3 – top feeding lake, V-6, V-7 – waterlogged shore of the low feeding lake, V-8 – middle course of the stream, V-9 – mouth reach of the stream, V-10 – terminal lake), measured in situ.

#### 4.4.4. Size fractionation of DOM

##### 4.4.4.1. DOC concentration pattern

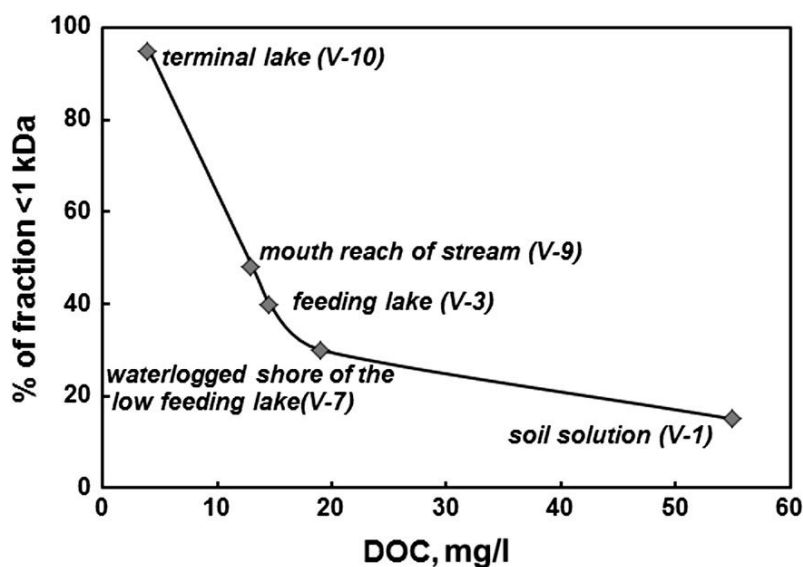
The relative proportion of various size fractions of DOC, from 100  $\mu\text{m}$  to 1 kDa for 5 representative samples (V-1, soil solution; V-3, humic feeding lake at the top of the watershed; V-7, waterlogged shore of another lake; V-9, mouth reach of the stream; V-10, terminal clear water lake) is listed in Table 4.3. In all samples, the mass fraction of LMW DOM (< 1–10 kDa) dominated the DOC with a significant proportion of HMW (10–100 kDa) colloids. It is important to note that the molar fraction distribution of different size organic components was dramatically different from that of the mass fraction. Since the molar weight is proportional to the third degree of molecular diameter, the difference in molecular mass between the association of molecules of 0.2  $\mu\text{m}$  diameter and a molecule of 10 kDa diameter (ca. 3 nm) reaches six orders of magnitude, being equal to  $8 \times 10^{-3}$  and  $8 \times 10^{-9} \mu\text{m}^3$ , respectively. As a result, the molar concentration of LMW fulvic acids was several orders of magnitude higher than that of the HMW

OM. The molar fraction and molar concentration of LMW (< 1–10 kDa) fulvic acids therefore dominated the DOC.

**Table 4.3.** DOC fractionation (%) for samples of Vostochniy: stream watershed (V-1 – soil solution, V-3 – top feeding lake, V-7 – waterlogged shore of the low feeding lake, V-9 – mouth reach of the stream, V-10 – terminal lake).

Fraction	V-1	V-3	V-7	V-9	V-10
100-10 $\mu\text{m}$	2.3	9.0	7.9	5.4	2.4
10-5 $\mu\text{m}$	0.23	0.60	25	1.3	2.0
5-0.8 $\mu\text{m}$	1.9	1.3	2.0	9.4	2.0
0.8-0.2 $\mu\text{m}$	57	1.9	12	3.2	-
0.2-0.1 $\mu\text{m}$	12	0.66	5.9	3.7	0.91
0.1 $\mu\text{m}$ -100 kDa	8.2	1.0	6.9	2.5	1.1
100-10 kDa	11	0.78	3.5	7.0	0.73
10-1 kDa	0.38	39	1.0	20	0.18
< 1 kDa	8.0	46	36	48	91

It can be seen from Fig. 4.5 that the LMW DOM fraction increased with downstream transit in parallel with a decrease in specific UV absorbance (Table 4.2). This would suggest that LMW molecules are linked to relatively low SUVA values, presumably because of low aromatic content. In contrast, the SUVA measurements of filtrates representing different molecular size fractions (Fig. 4.6) consistently showed that LMW DOM fractions were linked to high SUVA, e.g. high aromaticity. In fact, SUVA often increased sharply with decreasing filter pore size. This paradox stems from the huge difference in SUVA (and aromatic OM content) between soil solution V-1 (Fig. 4.6A) and oligotrophic lake V-10 (Fig. 4.6B). In fact, the lowest SUVA value in the oligotrophic lake (sample V-10) was independent of the pore size fraction. This strongly suggests that the main transformation of the LMW high SUVA fraction occurs between soil solution and first surface water reservoir, an intermediate small lake feeding the stream, or the mouth reach of the stream. In a large oligotrophic lake, the majority of the soil (humic) aromatic fraction of LMW is removed via autochthonous processes in the water column, presumably by photo- and biodegradation.

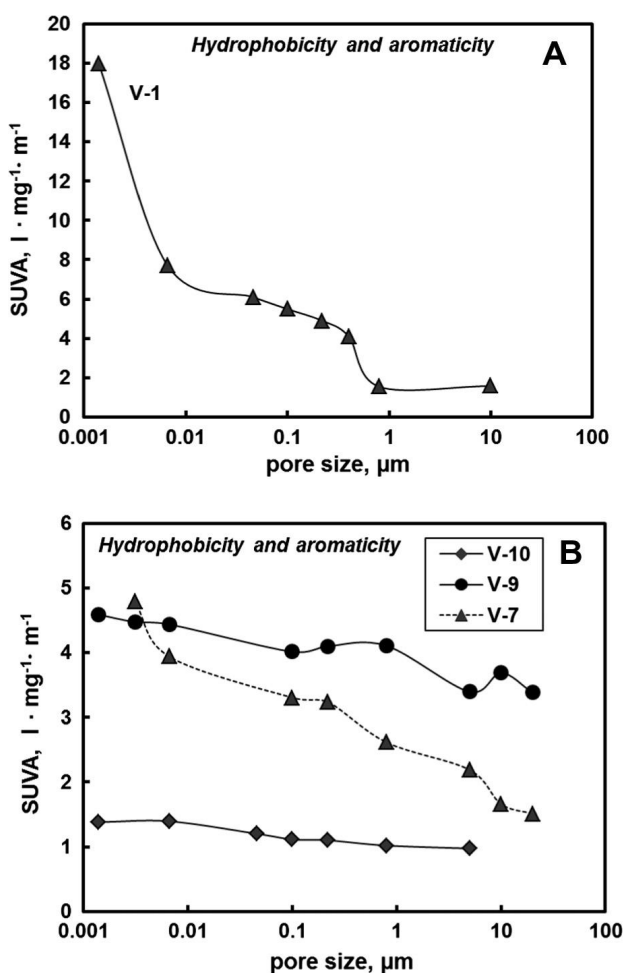


**Fig. 4.5.** Plot the% of < 1 kDa form as a function of DOC in 0.22  $\mu$ m fraction. The line is for guiding purposes.

Along the landscape profile of the watershed, the relative proportion of LMW (< 1 kDa) OC significantly increased from soil solution to stream and finally, to the terminal clear water lake, following the decrease in concentration of conventionally dissolved DOC<0.22 $\mu$ m (Fig. 4.5). The mouth reach of the stream (V-9) exhibited around 50% of DOC in < 1 kDa form, a typical value for other boreal landscapes (Guo et al., 2004; Prokushkin et al., 2011). The proportion of LMW < 1 kDa OC was also measured using equilibrium dialysis in 2008 and 2009; the results (25–55% of the LMW fraction for the intermediate samples of the Vostochniy stream) were in agreement ( $\pm$  5–10%) with the ultrafiltration results of 2009.

The DOM size fraction evolution in Fig. 4.5 is in agreement with previous observations of allochthonous vs. autochthonous OC, C/N ratio, hydrophobicity and aromaticity. It corresponds to progressive depolymerization of HMW soil humic acids, whether via heterotrophic aerobic bacterioplankton activity, as known for other boreal landscapes (Tranvik, 1988; Pokrovsky et al., 2011), or photodegradation in the feedstock lakes and stream channel (De Haan, 1993; Zuo and Jones, 1997; Wang et al., 2001; Albinet et al., 2010; Thorn et al., 2010). The increase in the proportion of bio-mineralized or photodegraded products of allochthonous HMW organic components may be responsible for the increase in the proportion

of LMW ligands in the continuum soil solution – feeding humic lake – stream – terminal oligotrophic lake. It can be hypothesized therefore that, after leaving the soil, the DOM is subjected to progressive degradation in stagnant water reservoirs. It is known from other boreal, permafrost-bearing aquatic systems that the longer the residence time of allochthonous DOM in the system, the higher the proportion of LMW products of photodegradation and bacterioplankton DOM transformation (Pokrovsky et al., 2011, 2013; Shirokova et al., 2013). An additional factor responsible for the trend in Fig. 4.5 may be summer phytoplankton activity, which produces LMW exometabolites dominating the speciation of OC in open water systems, especially in the large clear water lake.



**Fig. 4.6.** SUVA ratio with respect to molecular size fractions for samples (V-1, soil solution; V-3, top feeding lake; V-7, waterlogged shore of the low feeding lake; V-9, mouth reach of stream; V-10, terminal lake). The lines are for guiding purposes.

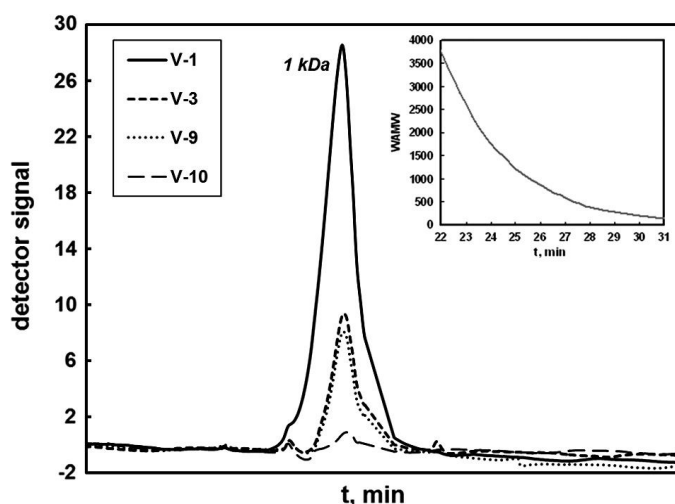
Therefore, we may tentatively attribute the increase in the LMW<1kDa fraction to the appearance of small-sized autochthonous OC in the form of phytoplankton exometabolites accompanied by the consumption of allochthonous soil-derived OM by heterotrophic



bacterioplankton. Like Siberian thaw lakes (Pokrovsky et al., 2011), in addition to exometabolites production, the higher proportion of LMW carbon in the largest lake can be a result of the decreasing input of soil and bog-derived OM to this lake, due mainly to a large water body in relation to the length of the shoreline or the watershed area. Therefore, the average residence time of the allochthonous organic macromolecules in these lakes is longer, exposing them to degradation by the bacterioplankton for a longer time (e.g. Amon and Benner, 1996 a, b). As a result, dominant organic macromolecules are smaller in size than mainly allochthonous DOM in small lakes located within the bog or the DOM of the forest stream. In a similar manner, the photooxidation of DOM is most pronounced in the clear water (oligotrophic) lake, notably due to much longer residence time of organic ligands in this lake. Given that photochemical processes degrade a part of the refractory pool of DOM that is not readily available to bacteria (Amon and Benner, 1996b), the importance of photodegradation in boreal lakes deserves further investigation.

#### *4.4.4.2. MW distribution from size exclusion chromatography*

Results from size exclusion chromatography (SEC) of the soil solution and stream water samples were similar and revealed a unimodal distribution (Fig. 4.7). In accord with the data on molecular size distribution, they demonstrated the absolute molar dominance of 1 kDa size compounds (Fig. 4.7, Table 4.2). Within the range 0.2  $\mu\text{m}$  to 10 kDa (2.8 nm) and a concentration of OC 5.2 mg/l, eight compounds of 5,200,000 Da were equivalent to the presence of  $4.4 \times 10^6$  molecules of 10 kDa. This strongly suggests a potentially significant importance of the LMW fraction in metal complexation reactions in boreal aquatic environments. The dominance of 1000 Da nominal molecular mass in the stream water is in agreement with results from most rivers of the Arctic Ocean basin (cf. Dittmar and Kattner, 2003 a, b).



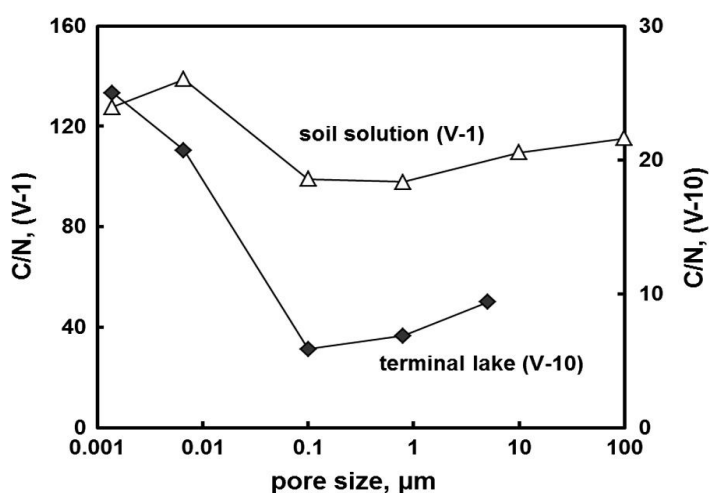
**Fig. 4.7.** Weight average molecular weight (WAMW) distribution in filtrates (0.22  $\mu\text{m}$ ) of samples (V-1 – soil solution, V-3 – top feeding lake, V-9 – mouth reach of the stream, V-10 – terminal lake), determined from size exclusion chromatography. Insert is a curve of WAMW distribution vs. time, calibrated using globular proteins.

Despite the dominant consensus that the bioavailability of OC decreases as its size decreases (Amon and Benner, 1996 a, b), with the LMW fraction in Arctic rivers being more refractory than the colloidal (1 kDa–0.45  $\mu\text{m}$ ) fraction (Guo and Macdonald, 2006), potential bioavailability of LMW OC may be still quite high. Indeed, the LMW complexes (< 1 kDa) of conventionally dissolved species are bioavailable in the case of passive diffusion through the biological membranes as the pore sizes of the transport channels of cell walls (10–30  $\text{\AA}$  in bacteria, 35–50  $\text{\AA}$  in plant cells; Carpita et al., 1979; Colombini, 1980; Trias et al., 1992) and that of the 1 kDa dialysis membrane (1–3 nm) are comparable. However, in situ biodegradation experiments with various size fractions of OM are necessary to constrain the bioavailability in boreal subarctic settings.

#### 4.4.4.3. C/N as an indicator of OM origin

The C/N ratio of the filtrates and ultrafiltrates showed a systematic variation for the two most contrasting samples, terminal oligotrophic lake and soil solution (Fig. 4.8). Compared with HMW fractions of 100 kDa–100  $\mu\text{m}$ , there was a 20–30% increase in C/N of 1–10 kDa

ultrafiltrates of soil solution V-1. For the oligotrophic lake, this increase from HMW to LMW fractions reached a factor of four to five. Such an evolution indicates a rather homogeneous composition of various size fractions of the soil solution and strongly suggests a sole source of DOM, presumably in the leachate of the plant litter subjected to minimal chemical and microbiological transformation corresponding to C/N of 100–140. In contrast, low C/N values (5–10) in the 0.1–10  $\mu\text{m}$  fraction of the large oligotrophic lake may stem from HMW phytoplankton and aquatic macrophyte exometabolites enriched in organic N vs. soil humus. The LMW fraction of the large lake had a C/N value between 20 and 25, approaching the value for the stream (45–50). The LMW soil fulvic acids and photodegradation and microbial degradation products < 10 kDa may also be present in this fraction, thereby contributing to the increase in C/N with the decrease in pore size. However, our data do not allow straightforward discrimination between the effect of allochthonous OM transformation and small sized phytoplankton exometabolites on the relative enrichment by organic N in the < 1–10 kDa fraction vs. HMW filtrates.



**Fig. 4.8.** C/N ratio with respect to molecular size fractions for samples. The lines are for guiding purposes.

#### 4.4.4.4. Optical characteristics of DOM

The color of the filtrates and ultrafiltrates progressively decreased from the 100  $\mu\text{m}$  to 1 kDa fraction in all the samples. Similarly, SUVA, reflecting the degree of hydrophobicity and aromaticity, significantly increased by a factor of 10 with the decrease in pore size for this

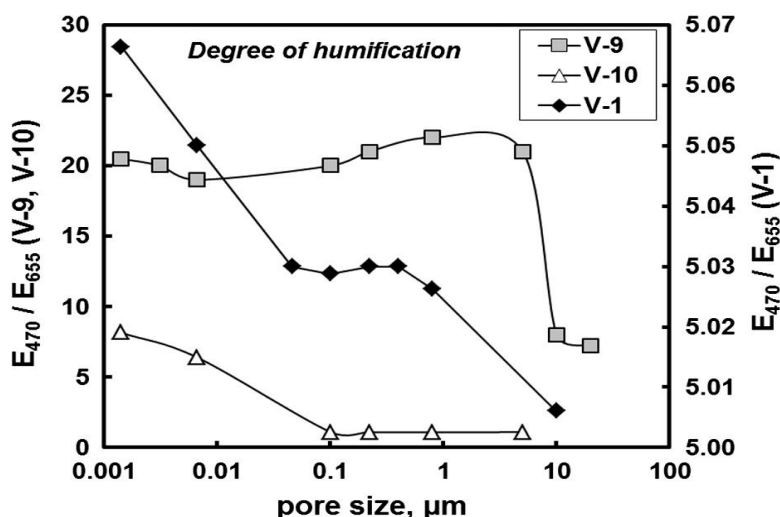
sample; the increase was much smaller for the low feeding lake V-7 (factor of 2.5) and was minimal ( $\leq 30\%$ ) in the stream V-9 sample and the terminal clearwater lake V-10 sample (Fig. 4.6A and B). These observations suggested that the transformation of DOM via microbial degradation and photodegradation was much more pronounced for the large lake and stream water than for the soil solution. This transformation is capable of modifying the distribution of carbon among different size fractions, presumably via decreasing the proportion of the hydrophobic/aromatic fraction in the LMW colloidal pool.

The ratios of the absorbance at different wavelengths in the UV/visible range were investigated to help reveal the basic features of OM chemical and source-related fractionation among the different filtrates and ultrafiltrates. Ratios such as  $E_{254}/E_{204}$ ,  $E_{254}/E_{436}$ , or  $E_{250}/E_{365}$  have been reported to be useful in OM characterisation (Battin, 1998; Hur et al., 2006; Spencer et al., 2007; Li et al., 2009). For example,  $E_{254}/E_{365}$  was used as a surrogate for DOM average MW, with samples with relatively low values having relatively higher MW DOM (Peuravuori and Pihlaja, 1997; Barreto et al., 2003; Berggren et al., 2007; Hiriart-Baer et al., 2008; Guo et al., 2011). However, in the studied boreal waters,  $E_{254}/E_{365}$  remained rather constant (typically between 4.5 and 5.5) and with minimal variation between 1m size and kDa size filtrates and ultrafiltrates (not shown). Barreto et al. (2003) calculated a value of  $E_{254}/E_{365}$  close to 4 for all water samples from Lake Ipe (Brazil) indicating the fulvic nature of the DOC. According to Peuravuori and Pihlaja (1997), values of  $E_{254}/E_{365}$  of 4.5 and 5.7 correspond to an average MW of 3380 Da (Lake Savojarvi) and 1120 Da (Utsjoki river), respectively for the humic solutes in whole water samples. Similarly,  $E_{280}/E_{350}$ , which may approximate to the aromatic carbon content of DOM (Croue et al., 2000), exhibited a very constant distribution among the different size fractions, slightly increasing in the  $< 1$  kDa ultrafiltrates from the humic lake (V-3) and stream (V-9), see Table ESM-4.

An alternative parameter for helping estimate the relative composition of autochthonous (aquagenic) vs. terrestrial (soil) DOM is  $E_{254}/E_{436}$  (Battin, 1998; Jaffe et al., 2004; Hur et al.,

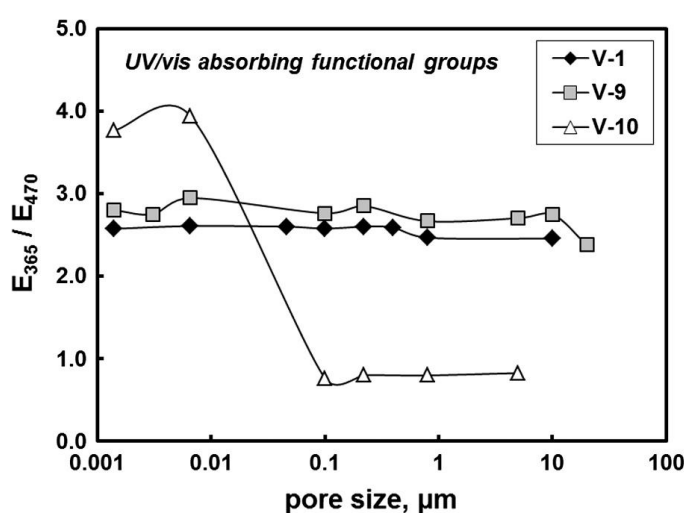
2006). For the majority of the samples this ratio ranged from 10–40, with the minimal values for soil solution V-1 and the maximal ones for the terminal lake V-10. The increase in  $E_{254}/E_{436}$  with decrease in pore size is consistent with the evolution of the C/N ratio in the filtrates/ultrafiltrates series.

The change in degree of humification, reflected in  $E_{470}/E_{655}$  (Chen et al., 1977; Schnitzer and Calderoni, 1985; Adani et al., 2006), for the filtration and ultrafiltration series is illustrated in Fig. 4.9 for soil solution, stream mouth and terminal lake. The soil solution exhibited a very weak increase (ca. < 5%) in  $E_{470}/E_{655}$  from the HMW to LMW fraction; the increase was a factor of four for the 10  $\mu\text{m}$ –1  $\mu\text{m}$  fractions of the stream water and a factor of eight for the 100 kDa–1 kDa fractions of the terminal lake. Like the evolution of the degree of hydrophobicity and aromaticity (see Fig. 4.6), the result suggests (i) a highly homogenous distribution of the degree of humification among different fractions of the soil solution V-1, containing exclusively allochthonous plant litter-derived humic material, and (ii) highly fractionated OM in the oligotrophic lake V-10 sample containing poorly humified fresh phytoplankton exometabolites of HMW (0.1–10  $\mu\text{m}$ ) and LMW allochthonous OM together with its microbial degradation and photodegradation products.



**Fig. 4.9.**  $E_{470}/E_{655}$  ratio, reflecting degree of humification, plotted as a function of pore size for samples. The lines are for guiding purposes.

Finally, the presence of the UV/visible absorbing functional groups, reflected in  $E_{365}/E_{470}$  (Uyguner and Bekbolet, 2005), showed no evolution during the cascade filtration and ultrafiltration of soil solution sample V-1 and stream water sample V-9 but significantly increased after 0.1  $\mu\text{m}$  filtration of terminal lake water sample V-10 (Fig. 4.10). Such a difference in ultrafiltration pattern of V-10 vs. other samples from the Vostochnyi watershed reflected the presence of at least two pools of DOM, the LMW allochthonous soil humic and fulvic acids ( $< 0.01 \mu\text{m}$ ), having elevated C/N ratio (cf., Fig. 4.8) and large size phytoplankton/macrophyte exometabolites.

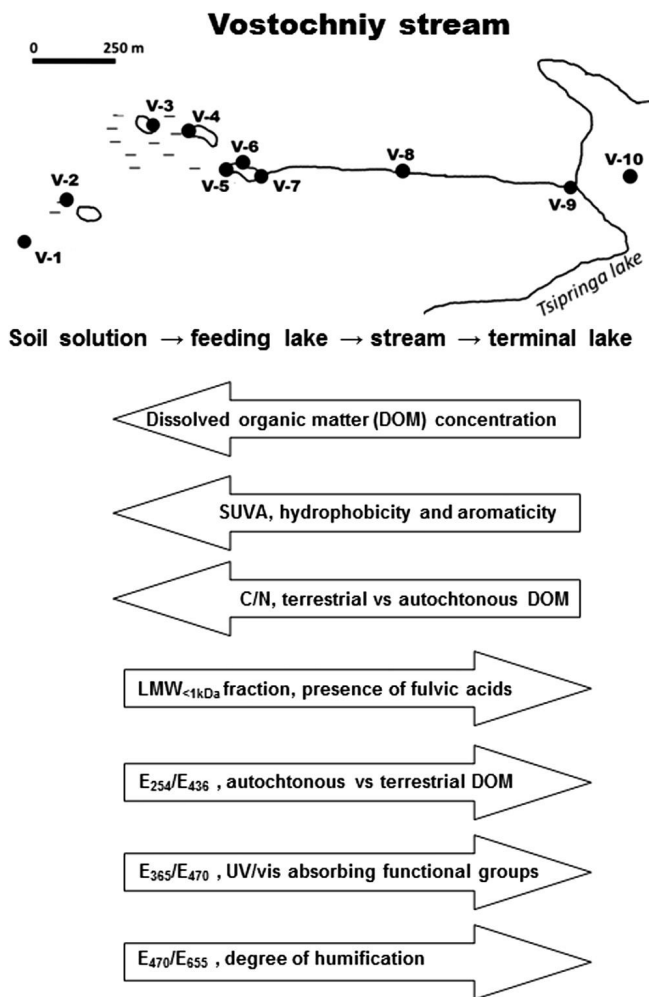


**Fig. 4.10.**  $E_{365}/E_{470}$  ratio, reflecting UV/vis absorbing functional groups, plotted as a function of pore size of filtrates and ultrafiltrates for samples. The lines are for guiding purposes.

## 4.5. Conclusions

The results demonstrate a significant and systematic change in basic chemical (DOC concentration, C/N ratio, SUVA and light absorbance ratios) and molecular size (100  $\mu\text{m}$ –1 kDa filtrates and ultrafiltrates) parameters of DOM in the continuum soil solution  $\rightarrow$  feeding bog and humic lakes  $\rightarrow$  stream  $\rightarrow$  terminal oligotrophic lake of a representative boreal subarctic watershed (as summarized in Fig. 4.11 and Table 4.4). These crucial changes in basic DOM parameters occurred over a very short distance of  $< 2 \text{ km}$ . For any type of water body, either lake or stream, the key parameter controlling the transformation of DOM in the water column is the water residence time. On one hand, it determines the relative effect of DOM input to the water

body from the surface flow or DOM in situ production by aquatic phytoplankton, periphyton and macrophytes. On the other hand, it controls the intensity of the DOM removal from the body, and DOM transformation via heterotrophic aerobic consumption or photo-degradation.



**Fig. 4.11.** Scheme of DOM parameter evolution along watershed profile, from soil solution to terminal lake.

As a result, the DOM evolution encountered in the Vostochniy watershed components reflects the complex process of the interplay between two main sources of DOC – soil humic and fulvic acids from the plant litter and bog water and aquagenic exometabolites of phytoplankton and macrophytes. Both sources are subject to biodegradation and photodegradation, the extent of which depends on the residence time of DOM in a given aquatic reservoir. Therefore, knowledge of soil type and the relative distribution of bogs within a small subarctic watershed are not alone

sufficient to predict the chemical and physical nature of the DOM that would be delivered by the stream to the ocean. Local hydrological regime, presence of intermediate stagnant water bodies and feeding lakes can significantly affect the original allochthonous signature of DOM during its transport from the soil solution/bog zone to the stream mouth. Moreover, the glacial or thermokarst origin of the hydrographic network distribution often suggests the presence of large, oligotrophic lakes as a terminus for small subarctic watersheds, in contrast to the estuarine mixing zone of large rivers. As such, small streams may deliver to the ocean DOC that is more significantly chemically fractionated among different size fractions and transformed by biodegradation and photodegradation compared with the DOM load of large rivers. This may be especially true for the most labile LMW<sub><1kDa</sub> fraction, by far dominant in molar concentration of boreal DOM and susceptible to travelling through the freshwater – seawater mixing zone without significant coagulation (cf. Dittmar and Kattner, 2003 a, b; Amon and Meon, 2004; Krachler et al., 2010).

**Table 4.4.** Summary of main parameters and their evolution in the continuum of the watershed from soil solution (V-1) to terminal oligotrophic lake (V-10).

Parameter	Meaning	V-1 → V-10	100 μm → 1 kDa
DOC	Dissolved organic carbon concentration	decrease	decrease
C/N	Terrestrial vs autochthonous DOM	decrease	increase
LMW	Presence of fulvic acids	increase	increase
SUVA	Hydrophobicity and aromaticity	decrease	increase
E <sub>254</sub> /E <sub>436</sub>	Autochthonous vs terrestrial DOM	increase	increase
E <sub>280</sub> /E <sub>350</sub>	Content of aromatic carbon	stable	stable
E <sub>254</sub> /E <sub>365</sub>	DOM average molecular weight	no trend	no trend
E <sub>365</sub> /E <sub>470</sub>	UV/vis absorbing functional groups	increase	increase
E <sub>470</sub> /E <sub>655</sub>	Degree of humification	increase	increase

To place this work in the context of permafrost thawing, one has to consider the difference in DOM transformation within small watersheds of the coastal zone and within the large Arctic rivers. It is possible that climate warming at high latitude will change the flux and the speciation of carbon in large rivers to a smaller degree than in small watersheds along the



Arctic Ocean coast. At present, the contribution of these small watersheds to the overall carbon flux from the land to the ocean is unknown (Holmes et al., 2012) but can be as high as 80% (Romankevitch and Vetrov, 2001). These small headwaters have been hypothesized to be the largest contributor to terrestrial DOC export per unit area (Ågren et al., 2007). In this regard, the major changes in DOC speciation delivered to the Arctic Ocean may occur within small watersheds having a significant proportion of glacial lakes or thaw ponds in their territories. Due to the relatively small discharge and baseflow regime during the Arctic summer, sufficient DOM residence time in these small water bodies, together with elevated surface temperature will stimulate production of phytoplankton, heterotrophic mineralization and photooxidation of allochthonous DOC (cf. Jansson et al., 2008; Porcal et al., 2009). In contrast, large subarctic rivers will be mostly affected by the increase in allochthonous soil OC input, due to the change in river discharge and the increase in the active layer thickness (Prokushkin et al., 2011; Bagard et al., 2011), as well as the increase in the winter discharge of terrestrial DOM (Stedmon et al., 2011). As such, further studies of small subarctic watersheds with high seasonal resolution are equally as important as for large rivers.

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### **Appendix A. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.orggeochem.2013.10.008>.

## References

- Adani, F., Ricca, G., Tambone, F. and Genevini, P., 2006. Isolation of the stable fraction, the core. of the humic acid. *Chemosphere* 65, 1300-1307.
- Ågren, A., Buffam, I., Jansson, M. and Laudon, H., 2007. Importance of seasonality and small streams for the landscape regulation of dissolved organic carbon export. *Journal of Geophysical Research* 112, G03003, 11 pp.
- Albinet, A., Minero, C. and Vione, D., 2010. Photochemical generation of reactive species upon irradiation of rainwater: negligible photoactivity of dissolved organic matter. *Science of the Total Environment* 408, 3367-3373.
- Alekhin, Yu.V., Ilina, S.M., Lapitsky, S.A. and Sitnikova, M.V., 2010. Results of a study of co-migration of trace elements and organic matter in a river flow in a boreal zone. *Moscow University Bulletin* 65, 380 – 386.
- Amon, R.M.W., Benner, R., 1996 a. Bacterial utilization of different size classes of dissolved organic matter. *Limnology and Oceanography* 41, 41–51.
- Amon, R.M.W., Benner, R., 1996 b. Photochemical and microbial consumption of dissolved organic carbon and dissolved oxygen in the Amazon River System. *Geochimica et Cosmochimica Acta* 60, 1783–1792.
- Amon, R.M.W. And Meon, B., 2004. The biogeochemistry of dissolved organic matter and nutrients in two large Arctic estuaries and potential implications for our understanding of the Arctic Ocean system. *Marine Chemistry* 92, 311-330.
- Archer, A.D. and Singer, P.C., 2006. An evaluation of the relationship between SUVA and NOM coagulation using the ICR database. *Journal of the American Water Works Association* 98, 110-123.
- Bagard, M.-L., Chabaux, F., Pokrovsky, O.S., Viers, J., Prokushkin, A.S., Stille, P., Rihs, S., Schmitt, A.-D. and Dupré, B., 2011. Seasonal variability of element fluxes in two Central Siberian rivers draining high latitude permafrost dominated areas. *Geochimica et Cosmochimica Acta* 75, 3335-3357.
- Barreto, S.R.G., Nozaki, J. and Barreto, J.W., 2003. Origin of dissolved organic carbon studied by UV-vis spectroscopy. *Acta Hydrochimica et Hydrobiologica* 31, 513-518.
- Battin, T.J., 1998. Dissolved organic materials and its optical properties in a blackwater tributary of the upper Orinoco River, Venezuela. *Organic Geochemistry* 28, 561– 569.
- Berggren, M., Laudon, H. and Jansson, M., 2007. Landscape regulation of bacterial growth efficiency in boreal freshwaters. *Global Biogeochemical Cycles* 21, GB4002, doi:10.1029/2006GB002844.
- Bishop, K., Pettersson, C., 1996. Organic carbon in the boreal spring flood from adjacent subcatchments. *Environment International* 22, 535-540.
- Bose, P. and Reckhow, D. A., 2007. The effect of ozonation on natural organic matter removal by alum coagulation. *Water Research* 41, 1516-1524.

- Bouillon, S., Yambélé, A., Spencer, R.G.M., Gillikin, D.P., Hernes, P.J., Six, J., Merckx, R. and Borges, A.V., 2012. Organic matter sources, fluxes and greenhouse gas exchange in the Oubangui River (Congo River basin), *Biogeosciences* 9, 2045–2062.
- Bourgeois, S., Pruski, A.M., Sun, M.-Y., Buscail, R., Lantoiné, F., Kerhervé, P., Vétion, G., Rivière, B. and Charles F., 2011. Distribution and lability of land-derived organic matter in the surface sediments of the Rhône prodelta and the adjacent shelf, Mediterranean Sea (France): a multi proxy study. *Biogeosciences* 8, 3107–3125.
- Callahan, J., Dai, M., Chen, R.F., Li, X., Lu, Z. and Huang, W., 2004. Distribution of dissolved organic matter in the Pearl River Estuary, China. *Marine Chemistry* 89, 211-224.
- Carpita, N., Sabularse, D., Montezinos, D., Delmer, D., 1979. Determination of the pore size of cell walls of living plant cells. *Science* 205, 1144–1147.
- Chen, Y., Senesi, N. and Schnitzer, M., 1977. Information Provided on Humic Substances by E<sub>4</sub>/E<sub>6</sub> Ratios. *Soil Science Society of America Journal* 41, 352-358.
- Chin, Y.-P., Aiken, G. and O’Loughlin, E., 1994. Molecular Weight, Polydispersity, and Spectroscopic Properties of Aquatic Humic Substances. *Environmental Science and Technology* 28, 1853-1858.
- Colombini, M., 1980. Pore size and properties of channels from mitochondria isolated from *Neurospora crassa*. *The Journal of Membrane Biology* 53, 1432–1434.
- Croue, J.-P., Violleau, D. and Labouyrie, L., 2000. Disinfection by-product potentials of hydrophobic and hydrophilic natural organic matter fractions: A comparison between and low-and high-humic water, In *Natural Organic Matter and Disinfection By-Products*. S. Barrett, S. W. Krasner, and G.L. Amy, Eds. American Chemical Society Symposium 761, Washington, D. C., 139-153.
- Davies, J., Jenkins, A., Monteith, D., Evans, C. and Cooper, D., 2005. Trends in surface water chemistry of acidified UK Freshwaters, 1988-2002. *Environmental Pollution* 137, 27-39.
- De Haan, H., 1993. Solar UV-light penetration and photodegradation of humic substances in peaty lake water. *Limnology and Oceanography* 38, 1072-1076.
- Dittmar, Th. and Kattner, G., 2003. The biogeochemistry of the river and shelf ecosystem of the Arctic Ocean: a review. *Marine Chemistry* 83, 103-120.
- Dittmar, Th. and Kattner, G., 2003. Recalcitrant dissolved organic matter in the ocean: major contribution of small amphiphilics. *Marine Chemistry* 82, 115-123.
- Dupré, B., Viers, J., Dandurand, J.-L., Polve, M., Bénézet, P., Vervier, Ph. and Braun J.-J., 1999. Major and trace elements associated with colloids in organic-rich river waters: ultrafiltration of natural and spiked solutions. *Chemical Geology* 160, 63-80.
- Edzwald, J.K. and Tobiason, J.E., 1999. Enhanced coagulation: US requirements and a broader view. *Water Science and Technology* 40, 63-70.
- Espinoza, L.A.T., Haseborg, E., Weber, M., Karle, E., Peschke, R. and Frimmel, F.H., 2011. Effect of selected metal ions on the photocatalytic degradation of bog lake water natural organic matter. *Water Research* 45, 1039-1048.

- Evans, C., Monteith, D. and Cooper, D., 2005. Long-term increases in surface water dissolved organic carbon: observations, possible causes and environmental impacts. *Environmental Pollution* 137, 55-71.
- Guggenberger, G., Christensen, B.T. and Zech, W., 1994. Land-use effects on the composition of organic matter in particle-size separates of soil: I. Lignin and carbohydrate signature. *European Journal of Soil Science* 45, 449-458.
- Guo, L., Macdonald, R.W., 2006. Source and transport of terrigenous organic matter in the upper Yukon River: evidence from isotope ( $\delta^{13}\text{C}$ ,  $\Delta^{14}\text{C}$ , and  $\delta^{15}\text{N}$ ) composition of dissolved, colloidal, and particulate phases. *Global Biogeochemical Cycles* 20, GB2011. <http://dx.doi.org/10.1029/2005GB002593>.
- Guo, L., Semiletov, I., Gustafsson, Ö., Ingri, J., Andersson, P., Dudarev, O. and White, D., 2004. Characterization of Siberian Arctic coastal sediments: Implications for terrestrial organic carbon export. *Global Biogeochemical Cycles* 18, GB1036, doi:10.1029/2003GB002087.
- Guo, L. and Santschi, P.H., 1996. A critical evaluation of the cross-flow ultrafiltration technique for sampling colloidal organic carbon in seawater. *Marine Chemistry* 55, 113-127.
- Guo, X.J., Xi, B.D., Yu, H.B., Ma, W.C. and He, X.S., 2011. The structure and origin of dissolved organic matter studied by UV-vis spectroscopy and fluorescence spectroscopy in lake in arid and semi-arid region. *Water Science and Technology* 63, 1010-1017.
- Guo, Y., Wan, Z. and Liu, D., 2010. Dynamics of dissolved organic carbon in the mires in the Sanjiang Plain, Northeast China. *Journal of Environmental Science* 22, 84-90.
- Hagel, L., 2001. Gel-filtration chromatography. *Current Protocols in Molecular Biology* UNIT 10.9 (Suppl. 44), 1–32. <http://dx.doi.org/10.1002/0471142727.mb1009s44>.
- Hiriart-Baer, V.P., Diep, N. and Smith, R.E.H., 2008. Dissolved Organic Matter in the Great Lakes: Role and Nature of Allochthonous Material. *Journal of Great Lakes Research* 34, 383-394.
- Holmes, R.M., McClelland, J.W., Peterson, B.J., Tank, S.E., Bulygina, E., Eglinton, T.I., Gordeev, V.V., Gurtovaya, T.Y., Raymond, P.A., Repeta, D.J., Staples, R., Striegl, R.G., Zhulidov, A.V., and Zimov, S.A., 2012. Seasonal and annual fluxes of nutrients and organic matter from large rivers to the Arctic Ocean and surrounding seas. *Estuaries and Coasts* 35, 369-382.
- Huguet, A., Vacher, L., Saubusse, S., Etcheber, H., Abril, G., Relexans, S., Ibalot, F., and Parlanti, E., 2010. New insights into the size distribution of fluorescent dissolved organic matter in estuarine waters. *Organic Geochemistry* 41, 595–610.
- Hur, J., Williams, M.A. and Schlautman, M.A., 2006. Evaluating spectroscopic and chromatographic techniques to resolve dissolved organic matter via end member mixing analysis. *Chemosphere* 63, 387-402.
- Ilina, S.M., Poitrasson, F., Lapitskiy, S.A., Alekhin, Yu.V., Viers, J. and Pokrovsky O.S., 2013a. Extreme iron isotope fractionation between colloids and particles of boreal and temperate organic-rich waters. *Geochimica et Cosmochimica Acta* 101, 96-111.
- Ilina, S.M., Viers, J., Lapitsky, S.A., Mialle, S., Mavromatis, V., Chmeleff, J., Brunet, P., Alekhin, Y.V., Isnard, H., Pokrovsky, O.S., 2013b. Stable (Cu, Mg) and radiogenic (Sr,

- Nd) isotope fractionation in colloids of boreal organic-rich waters. *Chemical Geology* 342, 63–75.
- IPCC 2007. *Climate Change, The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. Averyt, M.M.B. Tignor, et al.), Earth. Cambridge University Press, p. 996.
- Jansson, M., Hickler, Th., Jonsson, A. and Karlsson, J., 2008. Links between terrestrial primary production and bacterial production and respiration in lakes in a climate gradient in subarctic Sweden. *Ecosystems* 11, 367-376.
- Jaffé, R., Boyer, J.N., Lu, X., Maie, N., Yang, C., Scully, N.M., Mock, S., 2004. Source characterization of dissolved organic matter in a subtropical mangrove-dominated estuary by fluorescence analysis. *Marine Chemistry* 84, 195-210.
- Kaiser, K., Guggenberger, G., Haumaier, L. and Zech, W., 2002. The composition of dissolved organic matter in forest soil solutions: changes induced by seasons and passage through the mineral soil. *Organic Geochemistry* 33, 307-318.
- Kalbitz, K., 2001. Properties of organic matter in soil solution in a German fen area as dependent on land use and depth. *Geoderma* 104, 203-214.
- Krachler, R., Krachler, R.F., von Kammer, F., Suphandag, A., Jirsa, F., Ayromlou, S., Hofmann, Th. and Keppler, B.K., 2010. Relevance of peat-draining rivers for the riverine input of dissolved iron into the ocean. *Science of The Total Environment* 408, 2402-2408.
- Lara, R.J., Rachold, V., Kattner, G., Hubberten, H.W., Guggenberger, G., Skoog, A. and Thomas, D.N., 1998. Dissolved organic matter and nutrients in the Lena River, Siberian Arctic: Characteristics and distribution. *Marine Chemistry* 59, 301-309.
- Laudon, H., Berggren, M., Agren, A., Buffam, I., Bishop, K., Grabs, Th., Jansson, M. and Kohler, S., 2011. Patterns and dynamics of dissolved organic carbon, DOC. in boreal streams: the role of processes, connectivity, and scaling. *Ecosystems* 14, 880-893.
- Li, A., Hu, J., Li, W., Zhang, W. and Wang, X., 2009. Polarity based fractionation of fulvic acids. *Chemosphere* 77, 1419-1426.
- Matilainen, A., Gjessing, E.T., Lahtinen, T., Hed, L., Bhatnagar, A. and Sillanpää, M., 2011. An overview of the methods used in the characterisation of natural organic matter (NOM). in relation to drinking water treatment. *Chemosphere* 83, 1431-1442.
- Maksimova, M., 1967. Inorganic and organic composition of major ions in rivers of Karelian coast of the White Sea (in Russian). *Gidrobiologicheskie issledovaniya na Karelskom poberezhie Belogo moray*. Nauka, Leningrad, 9-20.
- Minor, E. and Stephens, B, 2008. Dissolved organic matter characteristics within the Lake Superior watershed. *Organic Geochemistry* 39, 1489-1501.
- Neal, C., Lofts, S., Evans, C.D., Reynolds, B., Tipping, E. and Neal, M., 2008. Increasing iron concentrations in UK upland waters. *Aquatic Geochemistry* 14, 263-288.

- Onstad, G.D., Canfield, D.E., Quay, P.D. and Hedges, J.I., 2000. Sources of particulate organic matter in rivers from the continental USA: Lignin phenol and stable carbon isotope compositions. *Geochimica et Cosmochimica Acta* 64, 3539-3546.
- Peuravuori, J. and Pihlaja, K., 1997. Molecular size distribution and spectroscopic properties of aquatic humic substances. *Analytica Chimica Acta* 337, 133-149.
- Pokrovsky, O.S. and Schott, J., 2002. Iron colloids/organic matter associated transport of major and trace elements in small boreal rivers and their estuaries (NW Russia). *Chemical Geology* 190, 141-179.
- Pokrovsky, O.S., Dupré, B. and Schott, J., 2005. Fe–Al–organic Colloids Control of Trace Elements in Peat Soil Solutions: Results of Ultrafiltration and Dialysis. *Aquatic Geochemistry* 11, 241-278.
- Pokrovsky, O. S., Schott, J. and Dupre, B., 2006. Trace element fractionation and transport in boreal rivers and soil porewaters of permafrost-dominated basaltic terrain in Central Siberia. *Geochimica et Cosmochimica Acta* 70, 3239–3260.
- Pokrovsky, O.S., Viers, J., Shirokova, L.S., Shevchenko, V.P., Filipov, A.S. and Dupré, B., 2010. Dissolved, suspended, and colloidal fluxes of organic carbon, major and trace elements in the Severnaya Dvina River and its tributary. *Chemical Geology* 273, 136-149.
- Pokrovsky, O.S., Shirokova, L.S., Kirpotin, S.N., Audry, S., Viers, J. and Dupré, B., 2011. Effect of permafrost thawing on organic carbon and trace element colloidal speciation in the thermokarst lakes of western Siberia. *Biogeosciences* 8, 565-583.
- Pokrovsky, O.S., Shirokova, L.S., Kirpotin, S.N., Kulizhsky, S.P., Vorobiev, S.N., 2013. Effects of anomalous high temperatures on carbon dioxide, methane, dissolved organic carbon and trace element concentrations in thaw lakes in Western Siberia in 2012. *Biogeosciences Discussion* 10, 7257–7297. <http://dx.doi.org/10.5194/bgd-10-7257-2013>.
- Pokrovsky, O.S., Shirokova, L.S., 2013. Diurnal variations of dissolved and colloidal organic carbon and trace metals in a boreal lake during summer bloom. *Water Research* 47, 922–932.
- Porcal, P., Koprivnjak, J.-F., Molot, L.A. and Dillon, P.J., 2009. Humic substances-part 7: the biogeochemistry of dissolved organic carbon and its interaction with climate change. *Environmental Science and Pollution Research* 16, 714-726.
- Prokushkin, A.S., Pokrovsky, O.S., Shirokova, L.S., Korets, M.A., Viers, J., Prokushkin, S.G., Amon, R.M.W., Guggenberger, G. and McDowell, W.H., 2011. Sources and the flux pattern of dissolved carbon in rivers of the Yenisey basin draining the Central Siberian Plateau. *Environmental Research Letters* 6, 045212, doi:10.1088/1748-9326/6/4/045212.
- Reuss, J., Cosby, B. and Wright, R., 1987. Chemical processes governing soil and water acidification. *Nature* 329, 27-32.
- Romankevitch, E.A. and Vetrov, A.A., 2001. Carbon cycle in the Russian Arctic seas (in Russian). Moscow, Science, p. 302.

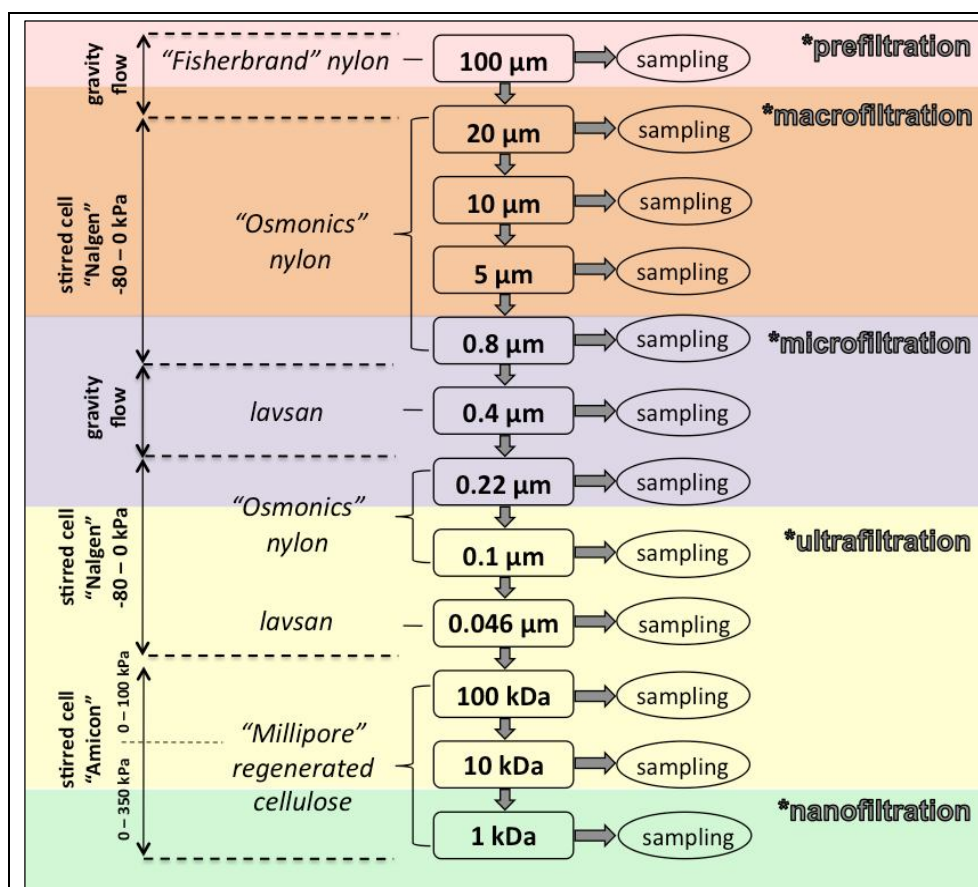
- Sachse, A., Henrion, R., Geldrecht, J., Steinberg, C.E.W., 2005. Classification of dissolved organic carbon (DOC) in river system: Influence of catchment characteristics and autochthonous processes. *Organic geochemistry* 36, 923-935.
- Sarkkola, S., Koivusalo, H., Laurén, A., Kortelainen, P., Mattsson, T., Palvivainen, M., Piirainen, S., Starr, M. and Finér, L., 2009. Trends in hydrometeorological conditions and stream water organic carbon in boreal forested catchments. *Science of The Total Environment* 408, 92-101.
- Schuur, E.A.G., Bockheim, J., Canadell, J.P., Euskirchen, E., Field, C.B., Goryachkin, S.V., Hagemann, S., Kuhry, P., Lafleur, P.M., Lee, H., Mazhitova, G., Nelson, F.E., Rinke, A., Romanovsky, V.E., Shiklomanov, N., Tarnocai, C., Venesy, S., Vogel, J.G. and Zimov, S.A., 2008. Vulnerability of permafrost carbon to climate change: Implications for the global carbon cycle. *BioScience* 58, 701-714.
- Schnitzer, M. and Calderoni, G., 1985. Some chemical characteristics of paleosol humic acids. *Chemical Geology* 53, 175-184.
- Selberg, A., Viik, M., Ehapalu, K. and Tenno, T., 2011. Content and composition of natural organic matter in water of Lake Pitkjärv and mire feeding Kuke River, (Estonia). *Journal of Hydrology* 400, 274-280.
- Selver, A.D., Talbot, H.M., Gustafsson, O., Boulton, S., and Dongen, B.E., 2012. Soil organic matter transport along an sub-Arctic river-sea transect. *Organic Geochemistry* 51, 63-72.
- See, J.H. and Bronk, D.A., 2005. Changes in C:N ratios and chemical structures of estuarine humic substances during aging. *Marine Chemistry* 97, 334-346.
- Shirokova, L.S., Pokrovsky, O.S., Kirpotin, S.N., Desmukh, C., Pokrovsky, B.G., Audry, S., Viers, J., 2013. Biogeochemistry of organic carbon, CO<sub>2</sub>, CH<sub>4</sub>, and trace elements in thermokarst water bodies in discontinuous permafrost zones of Western Siberia. *Biogeochemistry* 113, 573-593.
- Sihombing, R., Greenwood, P.F., Wilson, M.A., and Hanna, J.V., 1996. Composition of size exclusion fractions of swamp water humic and fulvic acids as measured by solid state NMR and pyrolysis-gas chromatography-mass spectrometry. *Organic Geochemistry* 24, 859-873.
- Skjelkvåle, B.L., Stoddard, J.L. and Andersen, T., 2001. Trends in surface water acidification in Europe and North America (1989-1998). *Water, Air, and Soil Pollution* 130, 787-792.
- Smith, R.E.H., Allen, C.D. and Charlton, M.N., 2004. Dissolved Organic Matter and Ultraviolet Radiation Penetration in the Laurentian Great Lakes and Tributary Waters. *Journal of Great Lakes Research* 30, 367-380.
- Spencer, R.G.M., Bolton, L. and Baker, A., 2007. Freeze/thaw and pH effects on freshwater dissolved organic matter fluorescence and absorbance properties from a number of UK locations. *Water Research* 41, 2941-2950.
- Spitz, A. and Leenheer, J., 2007. Dissolved organic carbon in rivers, *SCOPE* 42. *Biogeochemistry of major world rivers* 9, 1-21.

- Stedmon, C.A., Amon, R.M.W., Rinehart, A.J. and Walker, S.A., 2011. The supply and characteristics of colored dissolved organic matter, CDOM, in the Arctic Ocean: Pan Arctic trends and differences. *Marine Chemistry* 124, 108-118.
- Stevenson, F.J., 1994. *Humus Chemistry: genesis, composition, reactions*, 2nd ed., New York: Wiley, p. 496.
- Tamooh, F., Van den Meersche, K., Meysman, F., Marwick, T.R., Borges, A.V., Merckx, R., Dehairs, F., Schmidt, S., Nyunja, J. and Bouillon S., 2012. Distribution and origin of suspended matter and organic carbon pools in the Tana River Basin, Kenya. *Biogeosciences* 9, 2905–2920.
- Thorn, K.A., Younger, S.J. and Cox, L.G., 2010. Order of functionality loss during photodegradation of aquatic humic substances. *Journal of Environmental Quality* 39, 1416-1428.
- Tranvik L.J., 1988. Availability of dissolved organic carbon for planktonic bacteria in oligotrophic lakes of differing humic content. *Microbial Ecology* 16, 311-322.
- Tremblay, L. and Benner, R., 2006. Microbial contributions to N-immobilization and organic matter preservation in decaying plant detritus. *Geochimica et Cosmochimica Acta* 70, 133-146.
- Trias, J., Jarlier, V., Benz, R., 1992. Porins in the cell wall of mycobacteria. *Science* 258, 1479–1481.
- Twichella, S.C., Meyers, P.A., and Diester-Haass, L., 2002. Significance of high C/N ratios in organic-carbon-rich Neogene sediments under the Benguela Current upwelling system. *Organic Geochemistry* 33, 715–722.
- Uyguner, C. and Bekbolet, M., 2005. Implementation of spectroscopic parameters for practical monitoring of natural organic matter. *Desalination* 176, 47-55.
- Vasyukova, E.V., Pokrovsky, O.S., Viers, J., Oliva, P., Dupre, B., Martin, F. and Candaudap, F., 2010. Trace elements in organic- and iron-rich surficial fluids of the boreal zone: Assessing colloidal forms via dialysis and ultrafiltration. *Geochimica et Cosmochimica Acta* 74, 449-468.
- Vasyukova, E., Pokrovsky, O.S., Viers, J. and Dupre, B., 2012. New operational method of testing colloid complexation with metals in natural waters. *Applied Geochemistry* 27, 1226–1237.
- Viers, J., Dupre, B., Polve, M., Dandurand, J. and Braun, J., 1997. Chemical weathering in the drainage basin of a tropical watershed, Nsimi-Zoetele site (Cameroon): comparison between organic-poor and organic-rich waters. *Chemical Geology* 140, 181-206.
- Wang, G. S., Liao, C. H. and Wu, F. J., 2001. Photodegradation of humic acids in the presence of hydrogen peroxide. *Chemosphere* 42, 379-387.
- Wang, L., Wu, F., Zhang, R., Li, W. and Liao, H., 2009. Characterization of dissolved organic matter fractions from Lake Hongfeng, Southwestern China Plateau. *Journal of Environmental Sciences* 21, 581-588.



- Wolfe, A.P., Kaushal, S.S., Fulton, J.R. and McKnight, D.M., 2002. Spectrofluorescence of sediment humic substances and historical changes of lacustrine organic matter provenance in response to atmospheric nutrient enrichment. *Environmental Science and Technology* 36, 3217-3223.
- Zakharova, E., Pokrovsky, O.S., Dupre, B., Gaillardet, J. and Efimova, L., 2007. Chemical weathering of silicate rocks in Karelia region and Kola peninsula, NW Russia: Assessing the effect of rock composition, wetlands and vegetation. *Chemical Geology* 242, 255-277.
- Zuo, Y. and Jones, R. D., 1997. Photochemistry of natural dissolved organic matter in lake and wetland waters—production of carbon monoxide. *Water Research* 31, 850-858.

## ELECTRONIC SUPPORTING MATERIAL



**Figure ESM-1.** The scheme of cascade filtration used in this study.

**Table ESM-1.** Main filtration characteristics.

Pore size	Filer size	Filter material	Filter producer	Filtration pressure	Filtration unit
100 µm	300*300 mm	Nylon	Fisherbrand, USA	gravity flow	-
20 µm	Ø 37 mm	Nylon	Osmonics, GE, USA	-80 - 0 kPa	Nalgen, 250 ml
10 µm	Ø 37 mm	Nylon	Osmonics, GE, USA	-80 - 0 kPa	Nalgen, 250 ml
5 µm	Ø 37 mm	Nylon	Osmonics, GE, USA	-80 - 0 kPa	Nalgen, 250 ml
0.8 µm	Ø 37 mm	Nylon	Osmonics, GE, USA	-80 - 0 kPa	Nalgen, 250 ml
0.4 µm	2×100*250 mm	Lavsan	Dubna, Russia	130 - 150 kPa	-
0.22 µm	Ø 37 mm	Nylon	Osmonics, GE, USA	-80 - 0 kPa	Nalgen, 250 ml
0.1 µm	Ø 37 mm	Nylon	Osmonics, GE, USA	-80 - 0 kPa	Nalgen, 250 ml
0.046 µm	Ø 37 mm	Lavsan	Dubna, Russia	-80 - 0 kPa	Nalgen, 250 ml
100 kDa	Ø 76 mm	Regenerated cellulose	Millipore, USA	0 - 100 kPa	Amicon, 8400
10 kDa	Ø 76 mm	Regenerated cellulose	Millipore, USA	0 - 350 kPa	Amicon, 8400
1 kDa	Ø 76 mm	Regenerated cellulose	Millipore, USA	0 - 350 kPa	Amicon, 8400

**Table ESM-2.** DOC concentration, C/N and optical characteristics of filtrates and ultrafiltrates.

sample point	pore size, $\mu\text{m}$	DOC, mg/L	C/N	SUVA	E <sub>254</sub> /E <sub>436</sub>	E <sub>280</sub> /E <sub>350</sub>	E <sub>254</sub> /E <sub>365</sub>	E <sub>365</sub> /E <sub>470</sub>	E <sub>470</sub> /E <sub>655</sub>
OR-9	10	138.2	109.6	0.016	13.7	2.65	4.43	2.45	5.01
	0.8	135.2	97.8	0.015	13.5	2.65	4.43	2.47	5.03
	0.4	56.3	nd	0.041	14.2	2.68	4.46	2.59	5.03
	0.22	55.1	nd	0.049	14.5	2.70	4.50	2.60	5.03
	0.1	38.8	99.0	0.055	15.1	2.69	4.66	2.58	5.03
	0.046	34.2	nd	0.061	15.1	2.70	4.60	2.60	5.03
	0.0066	27.1	138.8	0.077	14.9	2.70	4.61	2.61	5.05
	0.0014	11.3	127.6	0.180	15.0	2.70	4.60	2.57	5.07
	OR-6	10	15.2	nd	0.044	9.8	2.60	4.13	2.37
0.8		14.9	nd	0.047	11.5	2.70	4.36	2.64	3.45
0.22		14.6	nd	0.042	16.5	2.90	4.86	3.40	5.74
0.1		14.5	nd	0.046	16.0	2.95	4.95	3.24	6.68
0.0066		14.3	nd	0.038	12.7	2.89	4.56	2.79	3.18
0.0031		14.2	nd	0.050	14.1	3.30	5.20	2.70	2.93
0.0014		7.7	nd	0.044	14.9	3.43	5.34	2.78	3.25
OR-2		20	17.9	nd	0.015	17.1	2.87	4.84	2.80
	10	17.1	nd	0.017	17.8	2.95	4.95	2.73	4.67
	5	17.0	nd	0.022	14.8	2.80	4.66	2.40	3.60
	0.8	16.3	nd	0.026	12.9	2.93	4.62	2.26	2.75
	0.22	16.1	nd	0.032	14.0	2.84	5.00	2.22	2.12
	0.1	15.0	nd	0.033	16.3	2.87	4.78	2.94	5.68
	0.0066	14.5	nd	0.039	13.9	2.81	4.61	2.50	3.78
	0.0031	13.3	nd	0.048	14.0	2.94	5.14	2.26	3.00
	OR-1	20	15.8	nd	0.034	16.5	3.07	5.17	2.38
10		15.0	nd	0.037	13.2	2.93	4.82	2.75	8
5		14.8	nd	0.034	12.0	2.68	4.38	2.70	21
0.8		13.3	nd	0.041	16.8	3.01	4.99	2.67	22
0.22		13.1	nd	0.041	14.5	2.85	4.71	2.85	21
0.1		12.9	nd	0.040	14.3	2.81	4.69	2.76	20
0.0066		11.8	nd	0.044	18.2	3.02	5.24	2.95	19
0.0031		10.7	nd	0.045	20.4	3.10	5.34	2.75	20
0.0014		7.6	nd	0.046	21.4	4.18	6.52	2.80	21
OR-8	5	5.3	9.4	0.010	nd	nd	nd	0.83	1.10
	0.8	5.2	6.9	0.010	nd	nd	nd	0.80	1.09
	0.22	5.1	nd	0.011	nd	nd	nd	0.80	1.10
	0.1	5.1	5.8	0.011	nd	nd	nd	0.76	1.11
	0.046	5.0	nd	0.012	nd	nd	nd	nd	nd
	0.0066	5.0	20.7	0.014	36.6	4.3	8.4	3.94	6.41
	0.0014	5.0	25.0	0.014	35.4	4.3	8.4	3.77	8.16

**Table ESM-3.** Weather characteristics for sampling periods for the data from meteorostation 22217 KANDALAKSHA (25m - 67 09N - 32 21E), [www.mundomanz.com](http://www.mundomanz.com)

Precipitation, mm	2007	2008	2009	2010	2011	2013
June	42.3	99.0	68.4	72.8	17.4	44.8
July	102.8	69.7	79.7	37.6	90.3	2.3
July 23 <sup>th</sup> 2009	-	-	0.0	-	-	-
August	38.8	99.3	105.5	65.0	54.1	-
Year	602.2	631.8	562.2	506.4	494.5	-
Temperature, °C	2007	2008	2009	2010	2011	2013
June	11.1	11.2	10.6	10.4	14.0	13.5
July	14.4	13.7	13.5	16.3	16.0	14.8
July 23 <sup>th</sup> 2009	-	-	18.0	-	-	-
August	14.0	10.7	13.4	11.9	11.8	-
Year	1.7	1.2	0.9	0.1	1.9	-

# Chapter 5

## **Conclusions and perspectives**

The podzol soil is characterized by low content of elements. The main reserves of organic substances in the soil are concentrated in the upper horizons. The formation of podzol on sandy and stony rocks contributes to migration of water-soluble compounds in the soil profile. The extent of movement of a metal in the soil system is related to the solution and surface chemistry of the soil and to the specific properties of the metal and associated waste matrix.

First part of this thesis was aimed at studying the influence of bacteria *Pseudomonas aureofaciens* on the sorption of zinc by different horizon of podzol soil. The experiments on the Zn adsorption onto podzol soil demonstrated a factor of 1.5 higher sorption capacity for the organic-rich horizon than for the mineral horizon, linked to the different complexation constants of Zn with the organic and mineral surface functional groups. There is an effect of the Zn adsorption decrease onto eluvial-humic horizon of podsol soil in the presence of live *Pseudomonas aureofaciens* (a factor of 2). In contrast, the presence of bacteria does not significantly modify the Zn adsorption onto mineral horizon. The main mechanisms of the decrease in the Zn adsorption onto the soil in the presence of the EPS-rich bacteria is shielding (protection) of the soil organic-rich particles by the relatively inert EPS. For example, the adsorption of EPS onto soil particles may lead to their aggregation, a decrease of the active surface area and, presumably, screening of the active surface centers from the aqueous  $Zn^{2+}$  ions. Special transmission electron microscopy and laser granulometry studies of soil–bacteria aggregates are necessary to confirm this mechanism.

These conclusions are important for remediation strategies, abiotically treated soils may be more efficient metal adsorbents than the native soil systems.

In the second part of this work, we investigated the release of various elements from different horizons of podzol soil in the presence of heterotrophic bacterium *Pseudomonas aureofaciens*. Dissolved organic carbon (DOC) concentrations decreased and dissolved inorganic carbon concentrations increased over the course of experiments with live bacteria due to ongoing DOC mineralization processes. Compared to abiotic systems, the observed decreases in the concentrations of major elements and many heavy metals that leach from the soil in the presence of bacteria have important consequences regarding our understanding of the role of bacteria in element mobilizations from soil to rivers. Presence of live bacteria enhanced the release of Fe, V, Rb, Ni, Cd, and Pb from eluvial-humic and illuvial horizons of podzol soil. In contrast, the release of K, Ca, Sr, Ti, Zn and Mn from soil decreased in the presence of bacteria, and Cu, Al, Ni, Mo and Cr release from soil was not affected by the presence of bacteria. The maximal effects of bacteria on divalent metals, such as Ni, Cd and Pb, may stem from the bacterial degradation of organic complexes of these metals in soil and their release into aqueous

solution. But it follows that chemical weathering in both organic and mineral horizons of podzol soil may not be strongly affected by heterotrophic bacterial activity; rather, the solution pH and DOC levels may control the intensity of element mobilization.

Last part of this thesis was aimed at studying natural organic matter size distribution in the natural waters in the continuum soil solution – bog – stream – and terminal lake of a representative boreal subarctic watershed. The results demonstrate a significant and systematic change in different parameters of DOM (DOC concentration, C/N ratio, SUVA, light absorbance ratios and molecular size) in the Vostochniy watershed components. Such an evolution reflects the complex process of the interplay between two main sources of DOC – soil humic and fulvic acids from the plant litter and aquagenic exometabolites of phytoplankton and macrophytes – both subjected to biodegradation and photodegradation, whose degree depends on the residence time of DOM in a given aquatic reservoir. Moreover, the glacial or thermokarst origin of the hydrographic network distribution often suggests the presence of large, oligotrophic lakes as a terminus for small subarctic watersheds, in contrast to the estuarine mixing zone of large rivers.

In order to better distinguish the influence of UV irradiation in organic matter transformation and the relative role of organic colloids in the binding of metals during migration from the soils and bogs to the river flow some experimental studies can be performed. It will allow quantifying the impact of photodegradation processes on organo-mineral colloids distribution.

Therefore, knowledge of soil type and the relative distribution of bogs within a small subarctic watershed are not alone sufficient to predict the chemical and physical nature of the dissolved organic matter that would be delivered by the stream to the ocean.