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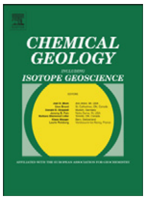

Microbial mobilization of cesium from illite: The role of organic acids and siderophores

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ABSTRACT

This paper aims to provide a mechanistic understanding of the mobilization of cesium (Cs) by bacteria from soil as part of a soil cleaning step. Whole metabolites and purified siderophores (pyoverdine, PVD), derived from the supernatant of a culture of *Pseudomonas fluorescens* (ATCC 17400), were supplied to illite spiked with either 10 or 100 $\text{mmol} \cdot \text{kg}^{-1}$ of Cs. The resultant effect was compared to low molecular weight organic acids (LMWOAs), citric and oxalic acids at 40 μM, or a synthetic chelant, i.e. acetohydroxamic acid (AHA) and desferrioxamine mesylate (DFOM) at 50 μM and 250 μM, respectively. In order to distinguish the direct (by ion exchange) from the indirect effects (by alteration of the illite crystal lattice) of purified PVD on the release of Cs, tetraethylammonium tetrafluoroborate (Me_4NBF_4) was used to evaluate the first mechanism, and the release of Fe from illite to evaluate the second. A model was subsequently developed to quantitatively describe the sorption phases on illite. A slight release of Cs from illite was observed with LMWOAs (7%) as well as with AHA and DFOM (9% and 22%, respectively, at 250 μM). The highest release was found with the purified PVD and a bacterial supernatant (43% and 40% in 7 days, respectively). Such a release of Cs from illite resulted from both: i) a likely direct mobilization of Cs from illite by means of ion exchange due to the positively charged carbon atom on the chromophore; and ii) an indirect mechanism due to the weathering of illite, as revealed in the chelating of iron from the illite crystal lattice by purified PVD. These results serve to demonstrate that Cs can be released to a significant extent from illite by *P. fluorescens*-producing chelating agents.

1. Introduction

Following nuclear accidents such as the one at Chernobyl and more recently Fukushima, radionuclides were dispersed into the air before being deposited on the soil within a 100-km radius around the damaged nuclear plant (Fujiwara et al., 2012). These radionuclides have contaminated many agricultural products, which raises the question of the future use of such soils. Due to the extent of the land surfaces affected, only in situ and inexpensive cleaning techniques are relevant. Such is the case with the phytoextraction method, provided that the soil/plant transfer process has been facilitated by increasing the phytoavailability of radionuclides. The major limitation of phytoremediation techniques does indeed stem from the slow pace of treatment as a consequence of the low availability of metals at a given point in time (Baker et al., 2000). On the whole, microorganisms such as bacteria play an important role in the biogeochemical cycling and (phyto)availability of metals and radionuclides (Brookshaw et al., 2012; Gadd, 2010; Uroz et al., 2009), by virtue of various processes (microbial respiration, changes in redox conditions and soil pH, production of ligands, etc.). In turn, these processes have led to mineral weathering, radionuclide chelation and dissolution, or even to the formation of new mineral phases (Dong and Lu, 2012; Dong et al., 2009; Gadd, 2010).

The recent Fukushima accident led primarily to contamination in radioactive cesium and iodine (Endo et al., 2012; Fujiwara et al., 2012; Kato et al., 2012). To date, only ^{137}Cs, with a half-life of 30 years vs. just 8 days for ^{131}I, remains a concern. Cs is known for its low mobility in the majority of soils (Cornell, 1993; Sawhney, 1972). In the Fukushima area, studies on the vertical migration of Cs have shown that 90% and 70% of the Cs were retained in the first 5 and 2 cm of the soil, respectively (Fujiwara et al., 2012; Kato et al., 2012). The main abiotic mechanism of Cs retention is adsorption. Cs was shown to be sorbed to micas and clays more strongly than to metal oxides, calcite or (alumino)silicate minerals such as quartz (Cornell, 1993) and it was also shown that fungi such as *Saccharomyces cerevisiae* were able to accumulate radioactive Cs competitively with minerals (Ohnuki et al., 2015). Owing to both their chemical characteristics (including their electronegativity) and layered structure (phyllosilicates), clays have a high specific surface area and feature the ability to absorb water; some specimens are even able to expand with a high cation exchange capacity (CEC). The importance of clay minerals to Cs behavior in soils or geological formations is in fact quite well known (Cornell, 1993; Komarneni, 1979; Sawhney, 1970) and can be quantitatively explained by a cation exchange process (Jacobs and Tamura, 1960; Sawhney, 1970). For high Cs concentrations, interactions are

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governed by planar sites present at the basal surface. Since these sites are non-specific, the Cs interaction is heavily influenced by both the major cation content in the water and the CEC value of the clay, which proves to be stronger for montmorillonite than for illite (Bradbury and Baeyens, 2000; Cornell, 1993; Nakao et al., 2008; Sawhney, 1972; Sawhney, 1970). The situation is different for trace concentrations of Cs. It is well known that the Cs interaction with soil particles, especially illite, is determined by specific sites. In addition to its association with non-specific basal surface sites, illite is known to have two other adsorption sites, namely: i) intermediate (type II) sites, and ii) "frayed edge sites" (or "FES") forming inner sphere complexes (Jacobs and Tamura, 1960; Sawhney, 1970). Illite FES show a particularly high affinity for Cs^+ ions, as compared to the planar sites of K^+-saturated vermiculite or montmorillonite (Sawhney, 1970; Staunton and Roubaud, 1997). In addition, FES and type II sites are predominantly accessible to cations with low hydration energies (i.e. K^+, Rb^+, NH_4^+), which can efficiently compete with Cs^+. This is one reason why mineral–Cs interactions are strongly influenced by the impact of microbes, resulting in the release of ammonium ions, thus aiding their metabolism (Brookshaw et al., 2012), or else by the addition of fertilizers to soils. These findings reflect a direct mobilization by means of competition through ion exchange. Another possible Cs mobilization would result in a modified stability of the mineral on which Cs is being sorbed due to mineral alteration (Brookshaw et al., 2012; Cornell, 1993), more specifically the weathering of clays by means of bacterial dissolution under the action of bacterial metabolites (Dong et al., 2009; Uroz et al., 2009; Wendling et al., 2005a, 2005b). This Cs mobilization by alteration corresponds to an indirect release.

These bacteria–mineral interactions therefore have the ability in direct and/or indirect ways as regards the mobilization and the dispersion of pollutant in the environment. Microbial metabolic activity actually gives rise to organic and inorganic compounds, such as humic substances, organic acids and several ions (nitrite, ammonium, carbonate and phosphate). These compounds may in turn influence radionuclide behavior in the soil. For example, the sorption of humic substances onto mineral surfaces leads to a new reactive interface offering different affinities for radionuclides, in comparison with the mineral surface itself (Bellenger and Staunton, 2008). These authors showed that the sorption capacity of Cs by clays can then decrease. In a field-scale study, the ion exchanges between microbial-released ammonium ions and Cs sorbed by lake sediments resulted in the seasonal release of Cs in the water column (Hinton et al., 2006). To date, although many studies have investigated the interaction of Cs with minerals and microorganisms, most of these have focused on global effects (Brookshaw et al., 2012; Newsome et al., 2014), and only a few of them describe the role of certain bacterial metabolites, e.g. organic acids and chelating agents such as citric and oxalic acids, EDTA, NTA and picolinate (Dumat and Staunton, 1999; Reinoso-Maset et al., 2013; Staunton and Roubaud, 1997; Wendling et al., 2005a, 2005b; Wendling et al., 2004). Therefore, the issue of direct vs. indirect Cs mobilization had not yet been clearly raised and, for the specific case of chelating agents, no molecules of bacterial origins (e.g. siderophores) had been used. This fundamental question is the central focus of the present paper. Well-controlled experiments were performed to study Cs desorption from illite for two Cs loadings starting at 10 and 100 mmol of stable Cs per kg of illite. The effect of purified pyoverdine (PVD) obtained from a culture of *Pseudomonas fluorescens* (ATCC 17400) was compared to that of commercial complexing agents (acetohydroxamic acid (AHA), desferrioxamine mesylate (DFOM)) as well as to that of citric (CA) and oxalic (OA) acids, used here as organic acids with chelating properties that were also produced by *Pseudomonas* bacteria. To distinguish the direct from indirect effects of purified PVD, tetraethylammonium tetrafluoroborate (Me_4NBF_4) was selected to compete with Cs. Moreover, a geochemical model was developed in conjunction with the experimental study in order to better understand the observed effects.

2. Materials and methods

2.1. Cs-spiked illite

Purified illite was used for purposes of this investigation (Illite du Puy $[(Si_{3.52}Al_{0.48})O_{10}(OH)_2]$ $z(Al_{1.17}Fe_{0.49}Mg_{0.33})(Ca_{0.04}Na_{0.12}K_{0.64})$ with CEC at about 12.7 $cmol \cdot kg^{-1}$, as characterized by Poinssot et al. (1999)). This illite specimen was analyzed by means of LA-ICP-MS-HR (laser ablation-inductively coupled plasma-mass spectrometry high resolution) for its background in Cs and Fe before spiking. Cs background and Fe original concentrations in the crystal lattice of illite are 0.5 $mmol \cdot kg^{-1}$ and 0.8 $mol \cdot kg^{-1}$ respectively. The illite was then spiked with 10 and 100 $mmol \cdot kg^{-1}$ of Cs. This order of magnitude for the concentration is similar to that employed by many authors, including Wendling et al. (2005b).

A 10 $g \cdot L^{-1}$ illite suspension was washed several times with a 1 mM NaCl solution and sterilized by tyndallization (3 cycles of 60 min at 70 °C, with 24 h of cooling at ambient temperature between each cycle) so as to eliminate microbial spores, with sterility being verified by plating 100 μL of illite suspension onto a solid Tryptic Soy Broth (TSB) medium, as well as to avoid illite alteration (Chen et al., 2014). The stock suspension was then spiked with 10 and 100 $mmol \cdot kg^{-1}$ of Cs and equilibrated for 48 h. Illite suspensions spiked with Cs were then centrifuged (7690 g for 15 min), washed twice with NaCl solution (1 mM) and then re-suspended into the same solution at 10 $g \cdot L^{-1}$. The supernatant was analyzed by ICP-MS to measure the non-sorbed Cs. The following batch experiments were conducted with 0.1 $g \cdot L^{-1}$ of spiked illite from aliquots of the solution at 10 $g \cdot L^{-1}$.

2.2. Pyoverdine production and purification

P. fluorescens (ATCC 17400) was used to produce PVD. The bacteria were first grown overnight at 28 °C in a TSB medium with shaking at 200 rpm. The bacterial cells were then centrifuged, washed twice with 9‰ NaCl solution and inoculated in a Casamino Acids (CAA) medium. CAA cultures were grown at 28 °C for 48 h, after which time the culture medium was centrifuged for 10 min at 7690 g, and a trichloroacetic acid solution (3%) was added to the supernatant to precipitate out proteins for 2 h under slow shaking conditions. After these 2 h, the supernatant containing PVD was filtered through a 0.7-μm Whatman filter paper and the pH of the filtrate was set at 8. Next, the PVD was purified by means of ion exchange chromatography using an Amberlite XAD-4 resin (Sigma), according to the method reported by Georgias et al. (1999). The PVD was eluted with methanol (100%) and then with diluted methanol plus H_2O (50:50 (*v*:*v*)), lyophilized and stored at −20 °C.

2.3. Bacterial mobilization of Cs from illite in batch experiments

2.3.1. Cs mobilization by LMWOAs and siderophore and siderophore-like molecules of either synthetic or bacterial origin

Various molecules were added to 0.1 $g \cdot L^{-1}$ of spiked illite in NaCl solution (1 mM) for 28 days at 28 °C. Subsequently, two siderophores were added at 50 μM and 250 μM: acetohydroxamic acid (AHA), a complexing agent; desferrioxamine mesylate (DFOM), a siderophore commercially