Evidence of plutonium bioavailability in pristine freshwaters of a karst system of the Swiss Jura Mountains

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Abstract

The interaction of trace environmental plutonium with dissolved natural organic matter (NOM) plays an important role on its mobility and bioavailability in freshwater environments. Here we explore the speciation and biogeochemical behavior of Pu in freshwaters of the karst system in the Swiss Jura Mountains. Chemical extraction and ultrafiltration methods were complemented by diffusive gradients in thin films technique (DGT) to measure the dissolved and bioavailable Pu fraction in water. Accelerator mass spectrometry (AMS) was used to accurately determine Pu in this pristine environment. Selective adsorption of Pu (III, IV) on silica gel showed that 88 % of Pu in the mineral water is found in +V oxidation state, possibly in a highly soluble $[\text{PuO}_2^+(\text{CO}_3)_n]^{m-}$ form. Ultrafiltration experiments at 10 kDa yielded a similar fraction of colloid-bound Pu in the organic-rich and in mineral water (18 % to 25%). We also found that the concentrations of Pu measured by DGT in mineral water are similar to the bulk concentration, suggesting that dissolved Pu is readily available for biouptake. Sequential elution (SE) of Pu from aquatic plants revealed important co-precipitation of potentially labile Pu (60 to 75 %) with calcite fraction within outer compartment of the plants. Hence, we suggest that plutonium is fully available for biological uptake in both mineral and organic-rich karstic freshwaters.

1. INTRODUCTION

Plutonium is an artificial element found in the environment mainly as a result of atmospheric nuclear weapons tests, nuclear accidents or authorized discharges (Alvarado et al., 2014; Hunt et al., 2013; Taylor, 2001). Pu represents a radiological risk if incorporated through the food chain because of its long biological half-life (Froidevaux et al., 2010a) and emission of high energy alpha-particles. Physico-chemical properties of plutonium species occurring in freshwaters define its radiological risk for aquatic biota and humans. Pu has complex redox chemistry and can be found under a variety of species in the environment. Consequently, it is challenging to accurately predict its biogeochemical behavior and bioavailability in aquatic ecosystems without precise knowledge of its physico-chemical form and speciation. Environmental Pu can be found simultaneously in both reduced (+III and +IV) and oxidized (+V and +VI) forms, with +IV and +V being the most common species in natural waters (Choppin et al., 1997; Xu et al., 2014). Pu (IV) is generally considered a low-mobility species, tending to precipitate into immobile phases, to form intrinsic colloids at significant concentration or to sorb onto organic and inorganic natural colloids at lower concentrations (Chawla et al., 2010; Novikov et al., 2006; Santschi et al., 2002; Utsunomiya et al., 2007). Nevertheless, field experiments have shown that Pu (IV) can be oxidized to Pu (V) in oxic conditions prevalent in the environment (Kaplan et al., 2004). Conversely, Pu (V) is much less sensitive to hydrolysis, has much lower partition coefficients to solid phase and has lower stability constants with organic ligands (*e.g.* with EDTA) (Katz et al., 1986). Thus, sorbed Pu (IV) can be remobilized as soluble and labile Pu (V) species, which then increase Pu transport and biouptake in aquatic ecosystems (Kaplan et al., 2004; Xu et al., 2014). In addition to the oxidative dissolution of Pu (IV), interactions with naturally-occurring organic matter have critical implications on its sorption and desorption kinetics in the subsurface water environments (Tinnacher et al., 2015).

The lability of Pu complexes with fulvic acid (FA) recently demonstrated in laboratory experiments provides new reasons for reconsidering the role of NOM for Pu bioavailability in natural freshwater environments (Cusnir et al., 2016; Cusnir et al., 2014).

Previous work on the use of the DGT technique to determine the lability of the Pu-FA complexes paved the way for direct *in-situ* measurements of the labile environmental Pu (Cusnir et al., 2014; Cusnir et al., 2015). A DGT device for Pu bioavailability measurements contains a polyacrylamide (PAM) gel assembly with a Chelex resin-gel as binding phase, protected with a filter (Davison and Zhang, 1994). The PAM gel layer enables the diffusion of bioavailable Pu species only, which are accumulated in the binding phase for subsequent analysis. Within reasonably short deployment time (2-3 weeks), DGT devices with a large surface area (*e.g.* 105 cm²) accumulate sufficient Pu for accelerator mass spectrometry measurements. Varying PAM gel thicknesses enables probing the dynamics of the molecular interactions of Pu with FA in natural waters *in-situ*, since the greater gel thickness increases the residence time and dissociation of Pu-FA complexes within the gel (Cusnir et al., 2016), thus contributing to the uptake of Pu. To predict the toxic effects of plutonium on aquatic organisms in the presence of dissolved NOM, the *in-situ* assessment of its bioavailability is required.

This paper reports on the use of the DGT and ultrafiltration techniques to determine the bioavailability of Pu in freshwaters of the karst system located in the Swiss Jura Mountains. A previous study on this system demonstrated a higher mobility of ²³⁹⁺²⁴⁰Pu compared to ²⁴¹Am and ¹³⁷Cs (Froidevaux et al., 2010b), suggesting that carbonate-rich vadose waters might contain Pu in a soluble, plutonyl-carbonate form similar to [UO₂(CO₃)_n]^{m-}. To determine the bioavailable Pu, we need to measure the fraction of total Pu that can be taken up by plant through a diffusion process of free and labile metal species (Davison, Nature 1994). Thus, we first used

ultrafiltration to reveal the fraction of Pu present as dissolved Pu species of size below 10 kDa. Pu in this fraction can be associated with small organic molecules such as FA. Because Pu-FA complexes were shown in a previous study to be labile in some extent ($0.2 < \xi < 0.4$) and have dissociation constant in the range of Co^{2+} and Fe^{3+} -FA complexes ($k_{\text{dis}} = 7.5 \times 10^{-3} \text{ s}^{-1}$), Pu-FA complexes will be measurable by the DGT technique. The concentration of Pu measurable by the DGT technique ($C_{\text{DGT}}(\text{Pu})$) has then be measured *in-situ* using large DGT devices for comparison with the concentration of Pu in the dissolved ($< 10 \text{ kDa}$) fraction. C_{DGT} is defined as the bioavailable fraction of Pu, *e.g.* the fraction that can diffuse through the plant cell wall.

Finally, a mass balance of Pu in plants was obtained using sequential elution that can access the carbonated fraction of Pu deposited on the plant biomass, the extra- and intra-cellular Pu fractions, and the Pu in the residue. This mass balance is necessary to compare the C_{DGT} concentration to the concentration of Pu in the intracellular fraction because the higher the C_{DGT} of Pu, the higher the intracellular Pu concentration is expected. These results will help to construct a comprehensive geochemical model of Pu mobility and bioavailability in carbonated mineral and organic-rich waters.

2. MATERIALS AND METHODS

2.1 STUDY AREA

To investigate the speciation of Pu in freshwaters, we chose two distinct aquatic environments located in the Swiss Jura Mountains (Fig. 1). In general, karst systems are characterized by high water permeability, with underground waters highly enriched in carbonates. Their Pu content comes only from the fallout of the atmospheric nuclear bomb tests

of the sixties, as demonstrated in a previous study by an average $^{238}\text{Pu}/^{239+240}\text{Pu}$ activity ratio of 0.031 ± 0.0089 ($n = 49$) (Froidevaux et al., 2010b).

The first aquatic environment was at the Venoge spring, which is located in the commune of L'Isle, about 20 km northwest of the city of Lausanne, and collects water from a karstic catchment area situated between 700 and 1400 m asl. The hydrological regime of the spring is mixed, with the highest flow rate in April-May (up to $7.50 \text{ m}^3 \text{ s}^{-1}$) and the lowest in the summer period (down to $0.01 \text{ m}^3 \text{ s}^{-1}$). The pH of the water varies between 6.5 and 7.5; at a temperature of ca. 7-8 °C, water is saturated with oxygen and CO_2 . Water of this mineral spring is oxic (Eh 490 mV NHE), carbonate-rich, and has a low NOM content (1 ppm at most). Detailed physico-chemical parameters of the water, as well as the precipitation regime during the sampling and field measurements campaign are given in the Supplementary Information (SI).

The second environment was the Noiraigue Bied brook, which is located at the Vallée-des-Ponts site at 1000 m asl. The complex watershed of the valley is formed by densely drained peat bogs and a river (Grand Bied), which collects atmospheric waters from the catchment and flows into the karstic sinkhole, giving rise to the Noiraigue spring at a distance of 4 km and an altitude of 736 m. The hydrological regime of the brook is strongly dependent on precipitation, with a lowest flow rate of $0.03 \text{ m}^3 \text{ s}^{-1}$ and a highest flow rate of $4.5\text{-}6.0 \text{ m}^3 \text{ s}^{-1}$. The pH of the water is ca. 6.5-7.5, the temperature varies from 0 °C in the winter season to +15 °C in the summer months. Water of the Noiraigue Bied brook is characterized by a high NOM content – up to 15-20 ppm. Detailed physico-chemical parameters of the water, as well as the precipitation regime during sampling and field measurements campaign are given in SI.

2.2 SAMPLE PREPARATION

All reagents used were of analytical grade from Merck, Fluka, or Sigma-Aldrich. Polyacrylamide gel sheets (6×22 cm) of 0.39 mm and 0.78 mm thickness and Chelex resin gels were provided by DGT Research Limited, Lancaster, UK. Local physico-chemical parameters of the water (pH, t °C, Eh, conductivity, dissolved oxygen) in the field were measured with a universal Multi 340i pocket meter equipped with a standard TetraCon[®] 325 conductivity cell and Cellox[®] oxygen sensor. NOM concentrations in water (calculated from measurement of total DOC) were quantified using UV-Vis spectrophotometry according to (Carter et al., 2012). WHAM 7 equilibrium model was used to calculate the chemical speciation of Pu in natural waters of the Noiraigue site, using the input data from Table SI-5.

2.2.1 WATER SAMPLES

To determine the Pu concentrations in water, we measured ^{239}Pu and ^{240}Pu using AMS (see § 3.4) in the bulk water samples from the Venoge spring and Noiraigue Bied brook (hereafter the activities are only given for ^{239}Pu). Water samples (10-50 L) were collected by pumping, filtered on line through a 0.5 μm Milligard[®] Nominal MCE cartridge and acidified on the spot with 1 mL conc. HNO_3 per L of water. Each sample was spiked with 1.7 pg of ^{242}Pu (source Pu242 Nr. 790 from LNSIRR), internal standard for AMS measurements. $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was dissolved in the water sample (0.25 g per 10 L) and Pu was co-precipitated with iron hydroxides at pH 8, by adding conc. NH_4OH . After decanting, the precipitate was dissolved in 5 M HCl and the solution heated to 80 °C under stirring to eliminate carbonates. 5 mL of a solution of Ca^{2+} of 100 mg mL^{-1} and 15 g of $\text{H}_2\text{C}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ were then added to the solution and calcium oxalate was precipitated at pH 2. After decanting and centrifugation, calcium oxalate was destroyed using microwave-assisted digestion (Milestone Ultraclave IV) in 20 mL of conc. HNO_3 (Luisier et al., 2009). 50 mg of $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ was added to the sample after digestion and

iron hydroxides were precipitated at pH 8. This precipitate was centrifuged and washed with deionized water ($<0.05 \mu\text{S cm}^{-1}$) and dissolved in 20 mL of 8 M HNO_3 for radiochemical separation of Pu. The radiochemical separation of Pu for AMS measurements was done by solid-phase extraction using AG 1-X4 anion exchange resin (chloride form) from Bio-Rad Laboratories Inc. as described by (Cusnir et al., 2015).

To determine the Pu (V) fraction in natural water, we used selective adsorption of Pu (IV) and Pu (VI) on silica gel as described by (Orlandini et al., 1986). Briefly, 100 g of silica gel (Fluka, 70-230 mesh) was added to 50 L of bulk water sample and left under vigorous stirring for 5 min. Afterwards the sample was decanted for 10 min and filtered to remove the silica gel. Pu (V) in the filtrate was spiked with ^{242}Pu , precipitated and analyzed as described above. Total Pu was measured separately in water from the same sampling batch and compared to Pu (V) concentration.

To determine the colloid-bound and dissolved fractions of Pu, 50 L water samples from the Venoge spring and Noiraigue Bied brook were submitted to cross-flow ultrafiltration through a 10 kDa Millipore Prep/Scale-TFF membrane (surface area 1 ft^2) to reach the concentration factor (cf) of 5. The retentate and permeate fractions were acidified, spiked with ^{242}Pu and submitted to radiochemical separation of Pu as described above.

2.2.2 AQUATIC PLANT SAMPLES

Aquatic mosses (mainly *Fontinalis antipyretica*) were collected in two locations at the Venoge spring (Fig. 1). Each sample was subsequently divided into two sub-samples for Pu sequential elution and measurements. The first location (L1) was in the close proximity to the Venoge spring's outlet, *ca* 20 m downstream from the outlet. The second (L2) was *ca* 50 m

down the stream, next to a 2-meter high waterfall. Vascular aquatic plants (*Phragmites australis*) were collected in one location in the Noiraigue Bied brook near the DGT samplers. Only plants completely covered by water were carefully collected. The sample was divided into two sub-samples for Pu sequential elution and measurements. Pu in these sub-samples was determined separately for leaves permanently exposed in water, and roots growing within the sediment.

Mosses and plants for sequential elution (Vazquez et al., 1999) of Pu were thoroughly washed with deionized water to remove any adhering sediment particles (Scheme SI-1). To determine the fraction of Pu co-precipitated with Ca-carbonates (most likely calcite) in aquatic mosses and vascular plants, the sample was leached in a citrate buffer (0.1 M citric acid anhydrous / 0.1 M sodium citrate tribasic dihydrate at pH 4.3) for 1 hour, then the plants were removed and the citrate solution filtered and set aside as the citrate fraction. Next, the plant sample was extracted with a 10 mM EDTA (pH 7) solution for one hour. The Pu in this EDTA solution was attributed to the extracellular Pu fraction. Subsequently, the plant sample was dried at 80 °C for 24 h to destroy cell walls and the intracellular Pu was eluted with 1 M HNO₃. Finally, the sample was dried, ashed, and the residual Pu was extracted in 8 M HNO₃ following a microwave digestion under pressure (50 bars) at 180°C. Between each elution step, the plant sample was rinsed with deionized water and the washouts were combined with the respective fraction. SE fractions were spiked with 1.7 pg of ²⁴²Pu and submitted to radiochemical separation for Pu. The calcium content in the citrate fraction was measured using ICP-OES. In addition, a sub-sample of plants was weighed, dried at 80 °C and ashed. Pu was extracted from the ashes using microwave-assisted digestion (Luisier et al., 2009). After microwave digestion the solution was filtered and submitted to radiochemical separation of Pu as described above to determine the bulk concentration of Pu.

2.3 DGTs

The fabrication and deployment of large surface (105 cm²) DGT devices for *in-situ* Pu bioavailability measurements were described and demonstrated by us earlier (Cusnir et al., 2015). To measure the bioavailable fraction of Pu in karstic freshwaters *in-situ*, we deployed large surface DGT devices of 0.39 mm and 0.78 mm gel layer thickness for 2 to 3 weeks in the two study sites. In the Venoge spring, DGTs were ballasted and suspended for deployment in the spring outlet. In the Noiraigue Bied brook, DGTs were deployed on a vertical support, at a 45 ° angle to the water flow. After deployment, the DGTs were disassembled and the Chelex resin-gel layers were retrieved for Pu elution. Each Chelex resin-gel sample was spiked with 1.7 pg of ²⁴²Pu internal standard and then 10 mL of 8 M HNO₃ was added. After 24 h elution, the samples were centrifuged, decanted and washed twice with 5 mL of 8 M HNO₃. The sample solutions were filtered through glass-fiber 0.45 µm Nalgene syringe filters when necessary and submitted to radiochemical separation of Pu for AMS measurements as described previously (Cusnir et al., 2015). The concentration of bioavailable (labile) Pu species in water C_{DGT} (µBq mL⁻¹) was calculated from the equation 1 (Cusnir et al., 2016; Warnken et al., 2007):

$$\frac{1}{A} = \frac{1}{C_{DGT} S t} \left(\frac{\Delta g}{D_M^{gel}} + \frac{\delta}{D_M^w} \right) \quad (1),$$

where A is the activity (µBq) of Pu accumulated in the binding phase (converted from the Pu concentration (at g⁻¹) measured by AMS), Δg is the diffusion layer (gel + filter membrane) thickness (cm), D_M^{gel} is the diffusion coefficient of Pu in the PAM gel (cm² s⁻¹, taken from (Cusnir et al., 2014), D_M^w is the diffusion coefficient of Pu in water ($D_M^w = D_M^{gel} / 0.85$), δ the diffusive boundary layer (0.03 cm from (Warnken et al., 2007), S the diffusion area (cm²), and t the duration of deployment (s). We used average C_{DGT} determined with DGT devices of the same

resin-gel thickness for each deployment campaign. Similarly, to compare the C_{DGT} with total Pu in the bulk water, we used average Pu concentration, determined for each period of DGT deployments.

2.4 Pu MEASUREMENTS by AMS

The AMS measurements of the Pu isotopes were performed at ETH Zurich using the low energy system “Tandy” (Christl et al., 2013). The compact (lab-sized) AMS system is ideally suited for determining ultra-trace amounts of actinides because it combines a high sensitivity (the transmission for actinides is up to 40%, (Vockenhuber et al., 2013) with an efficient suppression of neighboring masses (the abundance sensitivity is at a level of 10^{-12} for the actinides) (Christl et al., 2015). With the current setup of the AMS system, detection limits in the sub-femtogram range are reached for Pu isotopes (Dai et al., 2012). Details of the AMS measurement of Pu isotopes are described in (Christl et al., 2013). Briefly, the xPu-isotopes (x=239, 240, and 242) were measured sequentially (typically 10s, 20s, and 6s, respectively). The isotopic ratios of interest (here $^{239}\text{Pu}/^{242}\text{Pu}$ and $^{240}\text{Pu}/^{242}\text{Pu}$) were calculated from the repeated sequential measurement of samples, standards, and blanks. A possible interference of ^{238}U ions with ^{239}Pu (caused by “tailing” of ^{238}U) was carefully monitored and turned out to be negligible for all Pu-samples of this study. Each Pu-sample was measured between 1h and 1½h in total. The measured Pu-ratios were normalized to the ETH in-house standard “CNA” (Christl et al., 2013). The uncertainty of the reported Pu concentrations includes counting statistics and the variability of the measured isotopic ratios during the repeated measurement (of samples and standards).

3. RESULTS

3.1 Pu CONCENTRATIONS IN BULK WATER

A total of fourteen bulk water samples (20-50 L) were measured, yielding an average $^{240}\text{Pu}/^{239}\text{Pu}$ isotopic ratio of 0.194 ± 0.014 ($n = 14$). This result demonstrates that the origin of the Pu in the karst system is the fallout of the atmospheric nuclear tests of the sixties. The concentration of ^{239}Pu in the Venoge spring ranged from 0.43 to $3.44 \mu\text{Bq L}^{-1}$ ($n = 10$), similar to concentrations determined previously with alpha-spectrometry ($1\text{--}7 \mu\text{Bq L}^{-1}$, (Froidevaux et al., 2010b). In one case (Venoge water), the $0.5 \mu\text{m}$ Milligard[®] Nominal MCE cartridge cellulose filter was ashed after 300 L of water was filtered through and the Pu measured; less than 10 % of total Pu was found associated with the particulate fraction. When compared to precipitation data, the lowest ^{239}Pu concentration ($0.43 \pm 0.03 \mu\text{Bq L}^{-1}$) was associated with a precipitation regime ca. 70 % of the monthly precipitation norm (MPN). Nevertheless, a relatively low concentration ($0.83 \pm 0.17 \mu\text{Bq L}^{-1}$) was also measured on the day following a major rain event (40 % of the total monthly precipitations), possibly due to a significant dilution. The highest ^{239}Pu concentration ($3.44 \pm 0.14 \mu\text{Bq L}^{-1}$) in the Venoge spring was measured when monthly precipitations were close to the MPN and about one week later following a flood event. A similar pattern of ^{239}Pu concentrations was observed for the Noiraigue Bied brook. The lowest ^{239}Pu ($0.39 \pm 0.02 \mu\text{Bq L}^{-1}$) was measured in water sampled during a dry summer month with only 59 % of the MPN. The absence of rainfall had a similar effect on the NOM content – it dropped from 15 ppm to 10 ppm during this dry period. Higher ^{239}Pu concentrations ($3.13\text{--}3.40 \mu\text{Bq L}^{-1}$) were measured in water sampled during periods with a precipitation regime closer to normal (MPN 70-85 %) – this effect being probably due to the elution of ^{239}Pu deposited and retained in the organic-rich soil of the valley.

3.2 Pu SPECIATION IN NATURAL WATERS

The selective adsorption experiments enabled the determination of Pu (V) fraction in the natural water of the Venoge spring. We found 88 % ($n=2$, with a measurement uncertainty of 8 %) of the plutonium as ^{239}Pu (V) in water; this supports the hypothesis that in the carbonate-rich vadose waters the predominant form of Pu is a highly soluble $[\text{PuO}_2^+(\text{CO}_3)_n]^{m-}$, similar to uranyl-carbonate species. In the Noiraigue Bied brook water, it was not possible to carry out similar sorption experiments due to the high NOM content (15-20 ppm).

WHAM7 program contains parameters for Pu (IV) only, thus it was not applicable to the case of the Venoge waters, where more than 80 % of Pu was found as Pu (V). Nevertheless, in the case of the Noiraigue waters, the presence of a high concentration (about 15 ppm) of NOM will result in the reduction of Pu (V) to Pu (IV), as demonstrated in Cusnir et al. 2016, thus making calculation of the speciation of Pu (IV) possible. Varying FA and carbonate concentrations input in WHAM7 predicts that, at an elevated carbonate concentration of 150 mg L⁻¹ in the presence of 15 mg L⁻¹ FA, 72 % of Pu will be associated with carbonates at pH 6.5, while this fraction will increase to 94 % at pH 7. When carbonate concentration drops to 90 mg L⁻¹ at the same FA concentration of 15 mg L⁻¹, WHAM7 model predicts 48 % of Pu to be found associated to carbonates at pH 6.5 and 84 % at pH 7.0. Detailed output of WHAM7 calculations is given in the Table SI-8. Although NOM in high concentrations is expected to complex Pu, WHAM7 modelling shows that in carbonate-rich water at Noiraigue conditions Pu-carbonate complexes (very soluble and potentially bioavailable) are also being formed to a significant extent. As a general trend, increasing the FA concentration from 15 to 18 mg L⁻¹ slightly lowers the Pu-carbonate fraction at the same concentration of carbonate at both pH 6.50 and 7.00. Increasing the pH from 6.5 to 7.0 and CO_3^{2-} concentration from 90 to 150 mg L⁻¹ yields higher Pu-carbonate fractions.

3.3 Pu CONCENTRATIONS IN ULTRAFILTERED FRACTIONS

The results of the measurements of the ^{239}Pu in the ultrafiltered fractions of water sampled in the Venoge spring and the Noiraigue brook showed that most of the Pu is dissolved, as found in the fraction < 10 kDa (Fig. 2). The choice of a rather high cutoff (10 kDa) for the ultrafiltration membrane was due to the very low level of Pu concentration found in pristine waters and the necessity to filter high volume (50 L) in a relatively short time (< 24 h) to avoid modifications in the chemical composition of water, and clogging of the membrane. With this limitation, ultrafiltration experiments will not concentrate FA and Pu-FA complexes in the retentate fraction, because FA was demonstrated in our work to be less than 3000 kDa (Cusnir et al., 2016). Nevertheless, we found a rather similar colloid-bound ^{239}Pu fraction in both organic-rich water from Noiraigue (25 %, $n=2$) and mineral water from Venoge (18 %, $n=3$). The full mass balance of ^{239}Pu as a performance characteristic of cross-flow ultrafiltration (CFUF) yielded 83-105 % recoveries ($n=3$) for the Venoge water and 76-84 % ($n=2$) for the Noiraigue water. These performances were considered satisfactory, taking into account the high uncertainty associated to the very low ^{239}Pu concentration in our environmental water samples and the fact that Pu tends to sorb onto the filter membrane (Chawla et al., 2010).

3.4 DGT MEASUREMENTS OF FREE AND LABILE Pu SPECIES

In total, we deployed 13 DGTs in the Venoge spring and 9 DGTs in the Noiraigue Bied brook during two field campaigns in April-September 2014 and April-June 2015. Detailed information of each deployment campaign is given in the SI. DGTs retrieved after the deployment did not show traces of biofouling, probably due to the low temperature recorded for these waters, and preserved the initial form and condition of the gel assemblies. Figure 2

compares the C_{DGT} of ^{239}Pu with total ^{239}Pu and dissolved phase $^{239}\text{Pu} < 10\text{kDa}$, measured in both Venoge and Noiraigue waters. The average C_{DGT} of the ^{239}Pu measured in the Venoge spring was $1.39 \pm 1.03 \text{ } \mu\text{Bq L}^{-1}$ ($n=4$) with a gel thickness of 0.39 mm and $1.51 \pm 1.04 \text{ } \mu\text{Bq L}^{-1}$ ($n=4$) with a gel thickness of 0.78 mm. These concentrations correspond well to the total ^{239}Pu measured in the bulk water during the deployment period ($1.33 \pm 0.24 \text{ } \mu\text{Bq L}^{-1}$, $n=4$) as well as with the dissolved ^{239}Pu in the permeate fraction $< 10 \text{ kDa}$.

DGTs deployed in the organic-rich waters of the Noiraigue Bied brook showed a different pattern of Pu accumulation. The C_{DGT} of the ^{239}Pu calculated for a gel thickness of 0.39 mm was $1.30 \pm 0.76 \text{ } \mu\text{Bq L}^{-1}$ ($n=3$), which made only 60 % of the total ^{239}Pu measured in the bulk water during the deployment period ($2.12 \pm 0.35 \text{ } \mu\text{Bq L}^{-1}$, $n=4$), while the C_{DGT} of the ^{239}Pu calculated for a gel thickness of 0.78 mm was $2.71 \pm 1.50 \text{ } \mu\text{Bq L}^{-1}$ ($n=2$). This value overestimates the free and labile fraction and is higher than total ^{239}Pu measured in the Noiraigue waters. DGTs with a gel thickness of 0.78 mm deployed in the Noiraigue Bied brook systematically accumulated similar amounts of Pu as DGTs with a gel thickness of 0.39 mm. According to Equation 1, this yields higher apparent concentrations of labile Pu(C_{DGT}) in the river water.

3.5 DISTRIBUTION OF Pu IN DIFFERENT COMPARTMENTS OF AQUATIC MOSSES AND PLANTS

The activity of the ^{239}Pu per g of bulk fresh weight of mosses from Venoge measured in location L1 was $6.20 \pm 0.06 \text{ } \mu\text{Bq g}^{-1}$ ($n=2$) – five times lower compared to mosses sampled in L2 ($31.2 \pm 0.3 \text{ } \mu\text{Bq g}^{-1}$, $n=2$). The major fraction of Pu (60 %) in plants at both locations was extracted with citrate buffer (Figure 3) and was possibly present within the calcite precipitated on

the surface of mosses. The activity of ^{239}Pu per mg of Ca measured in the calcite fraction varied slightly between the two locations. We found $3.20 \pm 0.03 \text{ } \mu\text{Bq}$ of ^{239}Pu per mg of Ca in mosses near the spring and $5.30 \pm 0.12 \text{ } \mu\text{Bq}$ of ^{239}Pu per mg of Ca in mosses sampled next to the waterfall.

Measurements of Pu in aquatic plants from Noiraigue revealed a distribution similar to that in the Venoge mosses. Figure 3 shows that the major fraction of Pu eluted from the leaves was concentrated within the calcite, even to a greater extent (75 %) compared to mosses from the Venoge spring (60 %). In the roots, we only measured ^{239}Pu in extra- and intracellular compartments, as well as in the residue. We found a comparable activity of ^{239}Pu per g of fresh weight (Fig. 3 and Table SI-7) in these three fractions.

4. DISCUSSION

4.1 COLLOID-BOUND PLUTONIUM IN WATER

The results of the ultrafiltration experiments show that a rather constant 15-20 % of the total Pu is associated with colloids above 10 kDa in both mineral and organic-rich karstic waters, with a rather similar colloid-bound ^{239}Pu fraction at the Noiraigue site (25 %, $n=2$) and at the Venoge site (18 %, $n=3$). Waters from the organic-rich agricultural soils and peat bogs of the Vallée-des-Ponts (Noiraigue study site) are enriched in NOM and NOM-complexed Fe-Ca colloids (Atteia and Kozel, 1997; Mavrocordatos et al., 2000). We characterized the NOM from Noiraigue earlier with size-exclusion chromatography (Cusnir et al., 2016). These results showed mostly the presence of fulvic acids less than 3 kDa in molecular weight. In this respect, ultrafiltration with a membrane cutoff of 10 kDa results in considering FA-Pu complexes as

dissolved species. In addition, the lower recovery yield for the Noiraigue ultrafiltration experiments might be due to a slight adsorption of Pu-FA material onto the filter membrane.

4.2 FREE AND LABILE C_{DGT} PLUTONIUM

At the Venoge site, DGT with gel thicknesses of 0.39 mm and 0.78 mm give similar C_{DGT} , which matches the total Pu concentrations. Therefore, under “Venoge conditions”, dissolved Pu exists mostly in a readily available form – likely a plutonyl carbonate complex measurable by DGT, *i.e.* fully labile inorganic complexes. According to (Novikov et al., 2006) a plutonyl-carbonate $[PuO_2^+(CO_3)_n]^{m-}$ form, similar to $[UO_2(CO_3)_n]^{m-}$, can be found in water at equilibrium with atmospheric CO_2 . Our results are in agreement with the hypothesis that, at the Venoge site, plutonyl-carbonate complexes are the predominant species.

In contrast, at the Noiraigue site WHAM7 predicts that carbonate and soluble FA are competing for Pu, with up to 50 % of Pu bound to FA at pH 6.50 and 18 mg L⁻¹ of FA (Table SI-8). DGTs with gel thickness of 0.78 mm accumulated more Pu than expected from the (simplest) DGT theory. While the diffusive flux of free metals is obviously greater through a thinner gel, this can be compensated by an additional flux of Pu from labile Pu-FA complexes in the thicker gels, where enough time is available for these complexes to dissociate (Levy et al., 2012). We have previously shown in laboratory experiments that DGTs with a diffusive layer thickness of 1.96 mm accumulated more Pu than expected in the presence of dissolved FA (Cusnir et al., 2016). More specifically, this study showed that larger gel thicknesses increased the lability degree of the Pu-FA complexes. Small FA molecules have been shown to diffuse into the PAM gel (Van der Veeken et al., 2010), as well as into plants (Nardi et al., 2002), while a greater diffusive layer thickness of a DGT sampler increases the diffusion time of Pu-FA complexes

within the gel. A relatively high dissociation constant of Pu-FA complexes, as measured by (Cusnir et al., 2016), contributes to free Pu species, available for accumulation in the Chelex binding phase. In addition, dissociation of the Pu-FA complexes within the gel and resin-gel domain might change the Pu speciation, *e.g.* re-oxidation of Pu (IV) to Pu (V), with an increase in D_{gel} for Pu (V) (Cusnir et al., 2016). The good agreement of DGT (0.78 mm) results and bulk Pu concentrations in the Noiraigue Bied brook clearly demonstrated that the Pu-FA complexes in these organic-rich waters are labile and thus fully available for biouptake. While it was not possible to determine experimentally the Pu (V) fraction in this water, we suggest that some Pu might still be found as a $[\text{PuO}_2^+(\text{CO}_3)_n]^{m-}$, taking into account that the physico-chemical conditions of these aquatic systems (see SI) are, except for the NOM content, similar.

4.3 DISTRIBUTION OF Pu IN AQUATIC PLANTS

The sequential elution of Pu from aquatic mosses and vascular plants showed that in permanently immersed plants, more than 60-75 % of the Pu is associated with a fraction extracted by a citrate buffer at pH 4.3, most likely calcite, precipitated on the plant leaves. Only some 5 to 10 % of the total Pu is found in the intracellular fraction. Therefore, in these calcareous waters, calcite precipitation out-competes a direct biouptake of Pu by the plant cells. In the case of the Venoge spring, the Pu/Ca ratio in the water is roughly $0.01 \mu\text{Bq Pu mg}^{-1} \text{ Ca}$, whereas in the calcite precipitates on the mosses Pu is enriched, with a Pu/Ca ratio of approximately $1 \mu\text{Bq Pu mg}^{-1} \text{ Ca}$. This demonstrates a relatively high affinity of Pu to co-precipitate in a carbonate phase. The extent of calcite precipitation at the Venoge site is clearly higher in location L2 compared to L1 due to CO_2 -degassing of the carbonate-rich water after falling down the cascade (see Fig. 1). Accordingly, the amount of Ca eluted with calcite in mosses sampled in L2 was three times higher, compared to mosses sampled in L1. The Pu

concentrations of the precipitated calcite were similar in the two locations or even somewhat higher in L2 (downstream), which shows that over this small distance the removal of Pu by calcite precipitation does not significantly lower the Pu concentration in the bulk water, despite the above mentioned affinity of Pu to co-precipitate in calcite. Whether this process is a quantitatively important sink for Pu over longer distances has not been assessed. The calcite co-precipitation of Pu in organic-rich water of the Noiraigue Bied brook most probably involves Pu as Pu-FA complexes. These Pu-FA complexes have been demonstrated in a previous study to be somewhat labile ($0.2 < \xi < 0.4$) with a dissociation constant k_{dis} of $7.5 \times 10^{-3} \text{ s}^{-1}$, similar to transition metals such as Co^{2+} and Fe^{3+} . Thus, the local environment on the plant surface (similar to the DGT gel) contributes to dissociation of the Pu-FA complex and this allows the release of free Pu and the formation of a solid carbonate phase. Loss of dissolved CO_2 due to degassing promotes the precipitation of calcite on aquatic plants, co-precipitating Pu, both in mineral and in organic-rich water. This co-precipitation constitutes the main input of Pu to aquatic plants biomass, with only minute amounts of Pu found in the intracellular fractions.

The ultrasensitive determination of trace Pu with AMS in a pristine environment has provided a model of Pu speciation in natural freshwaters. A tentative sketch of the biogeochemical behavior of Pu in a karstic spring environment is given in Fig. 4. With a rather constant colloid-bound fraction, Pu is found predominately in the dissolved phase in both bicarbonate-rich and organic-rich water. Although Pu (V) has been experimentally evidenced in the mineral water, its reduction to Pu (IV) obviously takes place in the presence of soluble NOM, which is competing for Pu with carbonate ligands as NOM concentration in water increases. The exact speciation of Pu in these conditions can only be estimated using modeling approach.

Nevertheless, the determination of Pu bulk concentration and Pu C_{DGT} concentration shows that Pu is probably fully labile in mineral and organic-rich karstic waters.

5. CONCLUSION

This work demonstrates the validity of the DGT technique for Pu bioavailability measurements *in situ*. The results of our study suggest that the low percentage of colloidal Pu and the good agreement between Pu bulk and Pu DGT concentrations are an indication that Pu is fully bioavailable in the studied freshwaters. The determination of Pu as fully labile species in the mineral Venoge spring using DGTs agrees well with the presence of soluble plutonyl-carbonate $[PuO_2^+(CO_3)_n]^{m-}$ species and with a low proportion of colloid-bound Pu in the water. The use of DGT to determine the Pu concentration in the organic-rich Noiraigue water demonstrates the possibility of Pu-NOM complexes to contribute to the bioaccumulation of Pu via dissociation. In addition, the co-precipitation of Pu mostly with the calcium carbonate fraction in aquatic plants suggest that Pu is under a chemical form similar to $[PuO_2^+(CO_3)_n]^{m-}$. DGTs applied for ultra-trace Pu measurements in pristine environments require highly sensitive analytical techniques and in this study were associated with an important uncertainty on measurements. However, this approach proves its potential in continuous monitoring of discrete inputs of Pu (*e.g.* Fukushima, La Hague) and in the environments contaminated with higher Pu concentrations (*e.g.* Irish Sea), even with less sensitive analytical tools. We suggest that DGT concentration should be used instead of bulk concentration to determine radioactive concentration ratios (CR) in aquatic ecosystems.

6. ACKNOWLEDGEMENTS

This work was funded by the Swiss National Science Foundation (grant n° 200021-140230) and by the Swiss Federal Office of Public Health (PF and PS).

Figure captions.

Figure 1. Study and sampling area.

Figure 2. Determination of Pu as bioavailable species. Total ^{239}Pu measured in the bulk water during deployment of DGTs, concentrations of free and labile ^{239}Pu species measured with DGTs (left scale) and percentage of ^{239}Pu in the dissolved phase measured by CFUF (right scale) in the Venoge waters (top) and Noiraigue waters (bottom). Results are the average of n measurements and uncertainties are the sum of the absolute uncertainty of each measurement divided by n .

Figure 3. Sequential elution of Pu from different compartments of aquatic mosses and plants. Activity of ^{239}Pu ($\mu\text{Bq g}^{-1}$) measured in mosses sampled at the Venoge spring (top, a and b) and in aquatic plants sampled at the Noiraigue Bied brook (bottom, c). Each result is the average of 2 measurements and uncertainties are the sum of the absolute uncertainty of each measurement divided by 2. Activity of ^{239}Pu in the bulk are failing to perfectly match with the sum of fractions in mosses (a and b) due to uncertainty of type B and heterogeneity of calcite depositions in the different subsamples.

Figure 4. Tentative sketch of the biogeochemical behavior of Pu in karstic freshwater environments. Under “Venoge conditions”, in bicarbonate-rich and Ca-rich waters with a low content of NOM, Pu is found mainly in a +V oxidation state as soluble plutonyl-carbonate complexes. With the increase of soluble FA concentration in water, such as in “Noiraigue conditions”, carbonate competes with FA for Pu. Furthermore, the Pu (V) is being reduced to Pu (IV), which forms labile Pu-FA complexes, available for bioaccumulation. Due to the release of CO_2 from the water, the precipitation of the calcite (involving the Pu as co-precipitate) is a major input of Pu to aquatic plants in both environments.

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