Video Article

Experimental Column Setup for Studying Anaerobic Biogeochemical Interactions Between Iron (Oxy)Hydroxides, Trace Elements, and Bacteria

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Abstract

Fate and speciation of trace elements (TEs), such as arsenic (As) and mercury (Hg), in aquifers are closely related to physio-chemical conditions, such as redox potential (Eh) and pH, but also to microbial activities that can play a direct or indirect role on speciation and/or mobility. Indeed, some bacteria can directly oxidize As(III) to As(V) or reduce As(V) to As(III). Likewise, bacteria are strongly involved in Hg cycling, either through its methylation, forming the neurotoxin monomethyl mercury, or through its reduction to elemental Hg°. The fates of both As and Hg are also strongly linked to soil or aquifer composition; indeed, As and Hg can bind to organic compounds or (oxy)hydroxides, which will influence their mobility. In turn, bacterial activities such as iron (oxy)hydroxide reduction or organic matter mineralization can indirectly influence As and Hg sequestration. The presence of sulfate/sulfide can also strongly impact these particular elements through the formation of complexes such as thio-arsenates with As or metacinnabar with Hg.

Consequently, many important questions have been raised on the fate and speciation of As and Hg in the environment and how to limit their toxicity. However, due to their reactivity towards aquifer components, it is difficult to clearly dissociate the biogeochemical processes that occur and their different impacts on the fate of these TE.

To do so, we developed an original, experimental, column setup that mimics an aquifer with As- or Hg-iron-oxide rich areas versus iron depleted areas, enabling a better understanding of TE biogeochemistry in anoxic conditions. The following protocol gives step by step instructions for the column set-up either for As or Hg, as well as an example with As under iron and sulfate reducing conditions.

Video Link

The video component of this article can be found at https://www.jove.com/video/56240/

Introduction

Understanding and predicting trace element (TE) mobility and biogeochemistry in the environment is essential in order to monitor, develop, and apply appropriate management decisions for polluted sites. This especially applies in the case of toxic TEs such as arsenic (As) and mercury (Hg). The fate and speciation of these TEs in soil or aquifers are closely related to physico-chemical conditions, such as Eh and pH, but also to microbial activities that can play either a direct role on speciation or an indirect role on mobility.

Indeed, some bacteria can directly oxidize As(III) to As(V) or reduce As(V) to As(III). This affects As toxicity, since As(III) is the most toxic form of As, and mobility, since As(III) is more mobile than As(V), which can readily adsorb to iron (oxy)hydroxides or organic matter[1,2]. Likewise, bacteria are strongly involved in mercury cycling, either through its methylation, mainly by sulfate and iron reducing bacteria[3,4], forming the neurotoxin monomethyl mercury (readily bioaccumulated in the food chain), or through its reduction to volatile elementary Hg (Hg°)[5].

Both As and Hg fates are also strongly linked to soil or aquifer composition, since compounds such as organic matter or iron (oxy)hydroxides can influence their sequestration and bioavailability. As(V) adsorbs well to iron (oxy)hydroxides[6], whereas Hg has a very high affinity for organic matter (OM; mainly for thiol groups) but also for colloidal iron or manganese (oxy)hydroxides in OM depleted environments[7,8,9,10,11].

Bacterial activities can then influence the fate of TEs adsorbed to (oxy)hydroxides or organic matter through the reduction of iron (oxy)hydroxides or the mineralization of organic matter. Direct iron reduction by bacteria is the dominant pathway for iron reduction in sulfur depleted zones[12,13], Fe(III) being used as a terminal electron acceptor, whereas indirectly, Fe(III) can be reduced to Fe(II) by sulfide formed by a bacterial sulfate reduction[14]. Moreover, the presence of sulfate can also modify Hg and As speciation through the formation of complexes such as thio-arsenates[15] with As or metacinnabar with Hg.

Thus, a better understanding of the impact of iron and sulfate cycling on the fate of TE, such as Hg and As, could help us to better manage contaminated sites and maintain soil and water quality. Data could also contribute to reinforcing existing metal-mobility models. Microbial Fe(III)-reduction[16,17,18] can cause the desorption of TE. Theoretically, the indirect reduction of iron (oxy)hydroxides by sulfide produced by the microbial reduction of sulfate could also impact TE mobility. However, the extent and kinetics of these reactions are generally studied in batch homogenous systems or batch microcosms[16,18,19,20]. The drawback of batch experiments is the lack of dissociation of the occurring phenomena; indeed, activity is based on and limited by the resources present in the batch and only gives a final result of the shifts in speciation and adsorption. Using a column approach enables the renewal of inflowing media and the monitoring of the fate of TE over time and space. These conditions are more realistic when compared to an aquifer, where real phenomena are closely linked to continuous percolation conditions. Moreover, heterogeneous iron (oxy)hydroxide occurrence in aquifer sediments is common[21,23], and the spatial changes in the mineralogical and chemical composition of the solid phases certainly drives microbial activities.

To elucidate the influence of these heterogeneities on geo-microbial phenomena and the fate of iron-associated TE, we developed a laboratory, a continuously-fed column representing a simplified model aquifer. The column is filled to create an iron-depleted zone at the column entrance and an iron-rich zone at the top. Regular sampling ports enable us to study each zone individually as well as interface-associated phenomena. An example of the application of this experimental device for the study of Hg fate and speciation is already available[24]. Here we give a detailed description of the experimental setup and a second example of its application focused on the behavior of As in contaminated aquifers.

Protocol

1. Experimental Preparation

1. Acid-wash all materials (glass, polytetrafluoroethylene (PFTE)) in contact with samples (5 days in 20% nitric acid (HNO_3) v/v) followed by 5 days in hydrochloric acid (HCl) 10% v/v). Rinse several times with ultra-pure water and dry under a laminar flow hood prior to use.
2. Use polyethylene gloves (or similar) and a fume hood for all steps involving chemicals.

2. Prepare Hg and As Spiked Amorphous Iron Oxides

1. Prepare approximately 20 g of ferryhydrate ($Fe(OH)_3$): dissolve 50 g of $FeCl_3$-$6H_2O$ in 500 mL of ultra-pure water (resistivity >18 MΩ cm^{-1}) under agitation in a glass reactor with a stainless-steel impeller or magnetic stirrer. Initial pH is <2.
2. **Manually add a solution of 10 M NaOH to precipitate ferryhydrate.**
   NOTE: Approximately 50 mL will be required to precipitate all the iron (oxy)hydroxides. Adjust the pH to 6 and maintain agitation for 1 h to stabilize.
   1. For Hg-spiked (oxy)hydroxides: prepare 10 mL of $HgNO_3$ at 10 g L^{-1} and add 350 µL to the (oxy)hydroxide solution.
      NOTE: This will yield a final Hg content in the wet (oxy)hydroxides of ~4 µg g^{-1} (oxy)hydroxides.
   2. For As-spiked (oxy)hydroxides: prepare 100 mL of As_2O_3[25] at 10 g L^{-1} and add 70 mL to the iron-oxide solution. This will yield a final As(III) content of ~70 mg/g (oxy)hydroxides.
3. Leave under agitation with a stainless-steel impeller or magnetic stirrer for 3 h and then centrifuge for 20 min at 2,000 x g. Remove supernatant and re-suspend the (oxy)hydroxides in 500 mL of ultra-pure water. Repeat the centrifugation and rinsing steps twice. Recover the humid (oxy)hydroxides (solids have a moisture content of 85-90% wt.) and store at 4 °C until use.
4. Sterilize humid Hg or As-spiked iron oxides by gamma radiation, with a minimum absorbed radiation dose of 25 kGy.
5. **Control Hg and As (oxy)hydroxides contents**
   1. Determine Hg contents of the pellet[26].
      NOTE: We found 3.90 ± 0.08 µg Hg g^{-1} solid. Thus, the total amount of mercury added to each column in the 18.3 g of iron oxides was 71.4 ± 1.51 µg.
   2. Determine As contents in the pellet. Use hot acid mineralization (8 mL of 5 N HCL for 4 h at 50 °C) and analyze by Atomic Adsorption Spectrometry (AAS).
      NOTE: We found 70 mg As g^{-1} solid. Thus, the total amount of As added to the column in the 18.3 g of iron oxides was ~1.3 g.

3. Prepare Silica Gel and Sand Matrix

NOTE: A loose silica gel matrix was used to stop the fine iron oxides migrating from the sand/iron oxide mixture under the water flow. The final gel matrix was 6% silica gel so as not to form a block but just to loosely aggregate the oxides.

1. Prepare a 10% silica gel mixture by heating 4 g of silica gel in 40 mL of a solution of 7% KOH on a hot plate, stirring with a magnetic stir bar until dissolved.
2. Add 60 mL of ultra-pure water then cool the solution to ~20 °C. Quickly titrate with diluted phosphoric acid (20 %) to pH 7.5. Then quickly mix the liquid silica gel with 320 g of sterile sand and the previously added 18.3 g of Hg-spiked or As-spiked iron oxides before it solidifies.
3. Break up the "jellified" mixture by mixing with a spatula and keep sterile before use in step 4.

4. Setup the Column

1. Use glass columns with a water jacket cooling system (internal volume = 400 mL, height = 30 cm, diameter 3.5 cm) and five silica septa set regularly (every 5 cm) along the columns to enable you to sample all along the column.
2. Cut PTFE tubing (PTFE int Ø 3 mm) to ensure sufficient length at the column inlet and outlet. Connect the inflow to peristaltic tubing, which in turn is connected to the water/medium supply.

3. Sterilize all materials (glass, tubing) by autoclaving (1 h at 110 °C).
4. Attach column vertically.
5. Connect the water jacket to a water cooling system to maintain an average temperature of 20 °C.
6. **Fill column from the top as follows:**
   - A layer of damp rock wool to avoid solid loss;
   - 320 g of sterile sand (Fontainebleau sand, D_{50} = 209 µm);
   - 320 g of sterile sand mixed with 18.3 g hydrated amorphous iron oxides spiked either with Hg or As (see step 1) and fixed in a 6% silica gel matrix (see step 2).
7. Attach column vertically and connect ascendant flow of continuously N_2 bubbled ultrapure sterile water at low velocity (~2 mL h^{-1}).
8. Cover the column with aluminum foil to protect from the light.

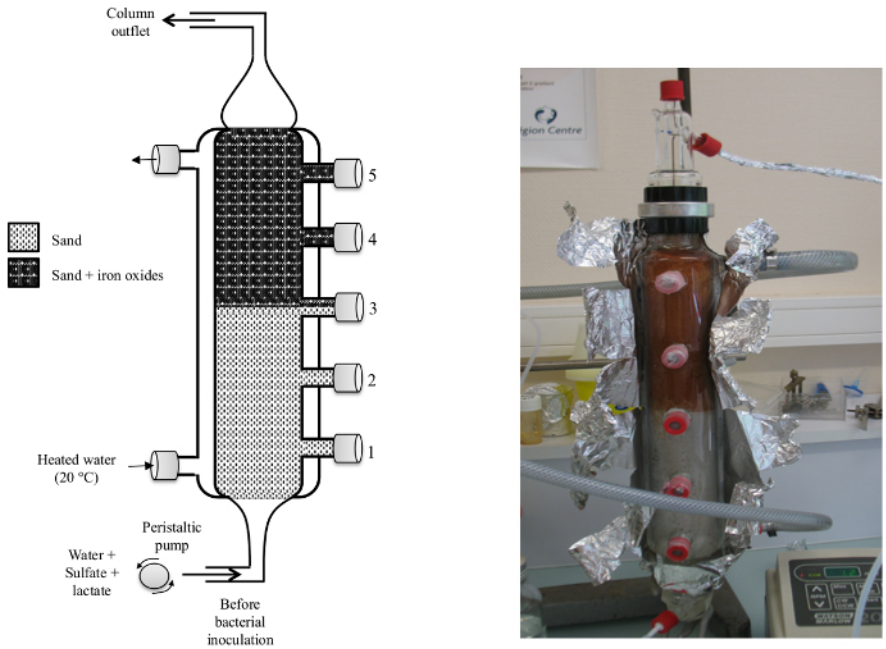

Figure 1: Sketch and photo of the column setup. Please click here to view a larger version of this figure.

Representative Results

Example 1. Impact of iron reduction of As mobility and speciation

The As column was directly inoculated with groundwater from a site presenting an As concentration higher than the drinking standards (Bracieux, Loire et Cher, France). Groundwater was sampled in sterile bottles, and stored at 5 °C until use. The column was fed from the bottom with this water containing the natural endogenous microbial community at a low flow-rate (2 mL h^{-1}) in order to facilitate bacterial attachment to the sand. Temperature was initially fixed at 25 °C in order to favor microbial growth and then decreased, after 54 days of continuous experiment, to 14 °C, which is the temperature of the aquifer. After the initial inoculation step, from day 0 to day 17, sulfate, lactate, and yeast extract (respectively 370 mg L^{-1}, 830 mg L^{-1}, and 250 mg L^{-1}) were introduced in the feeding water to activate sulfate bioreduction.

Example 2. Impact of bacterial iron and iron/sulfate reduction on Hg mobility and speciation

For this experiment, two columns were setup identically. The first was inoculated with an iron-reducing bacterial community and supplied with both molybdate (0.40 mmol L^{-1}), to inhibit sulfate reduction, and glucose, to favor iron-reducing bacteria (IRB column). Another column was inoculated with a sulfate-reducing bacterial community and fed with sulfate to create a sulfate reducing zone in the sandy lower half of the column as well as sodium lactate as a substrate (SRB column).

The two experimental vertical devices were fed from the bottom, first with sterile ultra-pure water, and then with groundwater that was sterilized by autoclaving (121 °C for 20 min). This groundwater was sampled in a chlor-alkali Hg contaminated site (referred to as Site X since the location is confidential). A peristaltic pump was used and the feeding flow-rate was set at 2.8 mL h^{-1}. Before inoculation, the columns were first rinsed for one week with ultra-pure water, a step during which total dissolved Hg ($[THg]_D$) and total dissolved iron ($[TFe]_D$) were monitored in the outflow. Next, columns were fed during one week with sterile Site X water to check the absence of abiotic mercury mobilization. The columns were then fed with Site X water amended with lactate and sulfate (370 mg L^{-1} of sodium sulfate and 830 mg L^{-1} of sodium lactate) for the SRB column, and with glucose and molybdate (10 g L^{-1} and 0.40 mmol L^{-1}) for the IRB column. After these preliminary abiotic steps, 20 mL of inoculum were injected into the inflowing water of each column on day 21.

Inocula were prepared by enriching the endogenous bacterial community from the Hg-contaminated Site X in specific culture media favoring either sulfate reduction or iron reduction. The preparation of both media was previously described[27]. Once sulfate and iron reduction were confirmed by measuring $[SO_4^{2-}]/S^{2-}]$ and [Fe(III)]/[Fe(II)] in these enrichments, they were used to inoculate the SRB and IRB columns, respectively.

Results from the mercury column experiments are available in Hellal *et al.* (2015)[24].

For this column experiment on arsenic mobility, the behavior over time of the concentrations in sulfate $[SO_4^{2-}]$, total dissolved iron (<0.45 µm) $[TFe]_D$, and total dissolved arsenic $[TAs]_D$ in the outlet solution are given in **Figure 2A**, and the evolution of these elements as well as pH and Eh along the column profile after 54 days of incubation are given in **Figure 2B**.

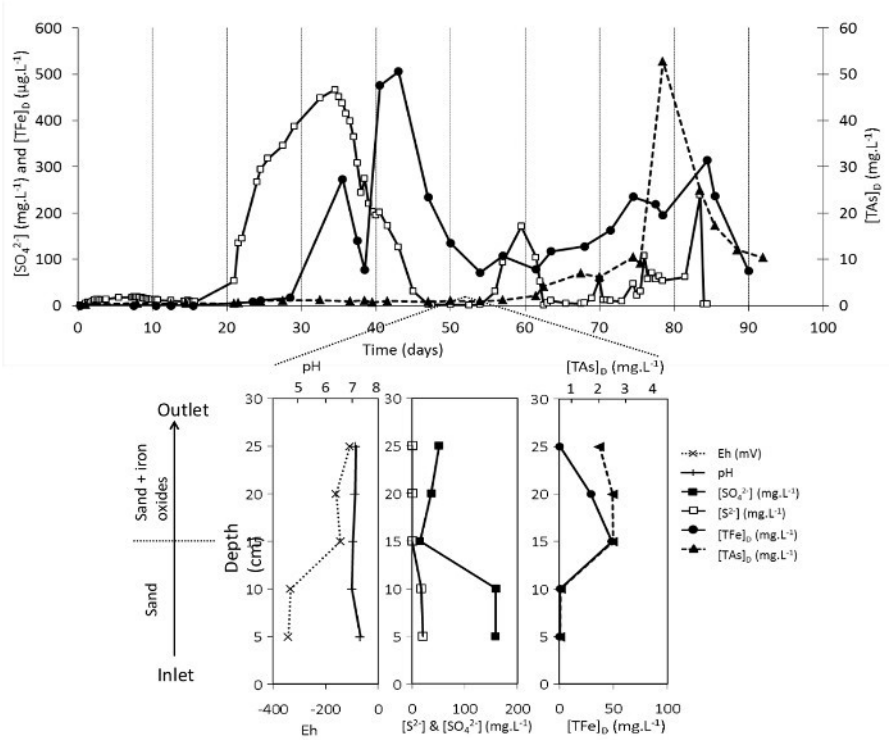

Figure 2. Monitoring the column. (**A**) Temporal evolution of $[SO_4^{2-}]$, $[TAs]_D$, and $[TFe]_D$ monitored at the column outlet. (**B**) Vertical column profiles for pH, Eh (Ref. Ag/AgCl), $[SO_4^{2-}]$, $[S^{2-}]$, $[TAs]_D$, and $[TFe]_D$ after 54 days of monitoring.

After two weeks of continuous experimentation with sulfate and lactate in the feed, a black-colored precipitate was observed at the interface between the two layers of sand (**Figure 3A**). This black zone progressively invaded the top iron (oxy)hydroxide-enriched zone of the column (**Figure 3B**). At the end of the experiment (day 95), the entire upper layer was black (**Figure 3C**).

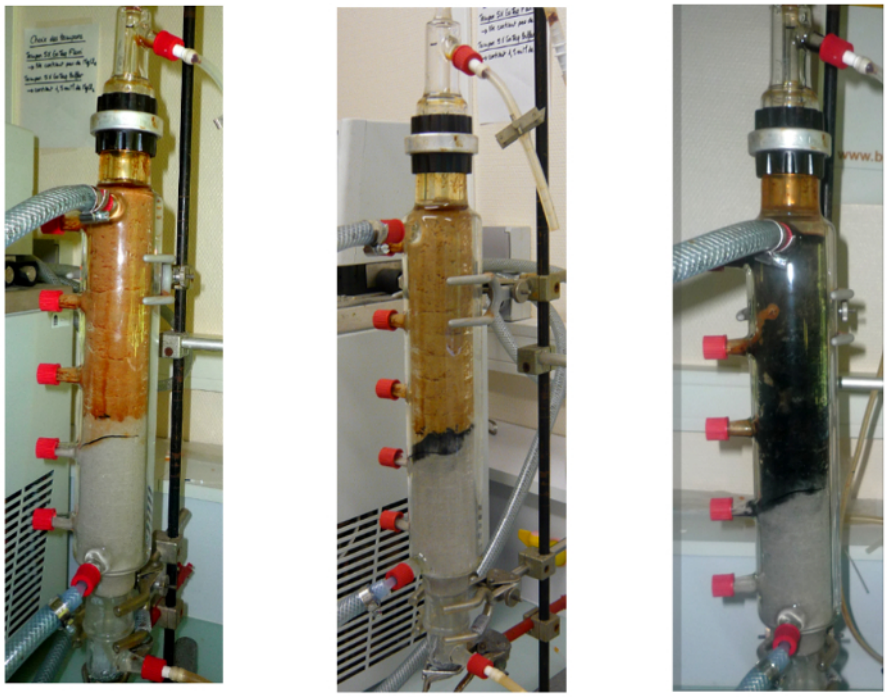

Figure 3: Changes in the aspect of the hydroxide zone into the column during the experiment.
(**A**) The black zone appeared at the interface (day 35), (**B**) the black precipitates progressively invaded the hydroxide zone (day 45), (**C**) the hydroxide zone was entirely black (day 65). Please click here to view a larger version of this figure.

After 35 days of continuous running, a decrease in [SO_4^{2-}] was observed at the column outlet, followed by a transient increase of $[TFe]_D$ in the 0.45 µm filtrated samples. From day 60, a significant rise of $[TAs]_D$ in the outlet water was measured. A profile of the physical and chemical parameters along the experimental system was obtained on day 54, when the sulfate-reduction was clearly active, by sampling through the 5 septa. The pH did not vary, remaining close to pH 7 (from 7.00 to 7.32) from the bottom to the top of the column. By contrast, the redox potential was clearly different in the two layers (**Figure 2B**), presenting values close to -400 mV (ref. Ag/AgCl) in the bottom, deprived of iron, and increasing to values close to -200 mV (ref. Ag/AgCl) in the top iron-rich zone. In the bottom layer, dissolved sulfide reached concentrations close to 20 mg L^{-1}, then decreased to values lower than 1 mg L^{-1} in the iron-rich zone. The sulfate concentration was globally lower in the column than in the feed water; however, it decreased sharply at the interface between iron-deprived and iron-rich zones. Arsenic was detected in the 0.45 µm filtered samples from the upper zone, that contained the As-spiked iron (oxy)hydroxides). Thio-arsenate species were detected close to the interface zone, and the intermediary product of sulfate-reduction; thiosulfate was present in the bottom iron-deprived layer[28].

The results of the sulfate and thio-arsenate concentration profiles indicated a peak of sulfate-reduction activity at the interface between iron-deprived and iron-rich layers. In the iron-rich layer, the most likely occurring processes should be Fe(III) reduction by dissolved sulfide to produce Fe(II), which would then precipitate with dissolved sulfide as the black FeS mineral[29]. Some arsenic initially bound to iron (oxy)hydroxides could have been mobilized by the Fe(III) reduction but then re-adsorbed onto the remaining iron (oxy)hydroxides as long as adsorption sites were available. As the black FeS front progressed upwards, the quantity of available adsorption sites decreased and arsenic concentration in the outlet water increased. The higher sulfate-reducing activity, measured near the iron-deprived and iron-rich interface, could be explained by the consumption of dissolved sulfide by iron; since the product issued from sulfate-reduction was being consumed, this reaction was energetically more favorable[30]. This phenomenon was observed thanks to the column setup.

Discussion

The experimental column setup proved to be a convenient laboratory device to study anaerobic biogeochemical processes in continuous conditions. Continuous column systems allow working in conditions closer to those of real aquifers than slurry batch systems or microcosms. Continuous systems can simulate the movement of groundwater through aquifer sediments.

The most critical step within the protocol is preparing the TE-iron (oxy)hydroxides and the mixture with silica gel and sand, which needs to be created quickly in order to obtain a homogenous texture. Beyond this general critical step, the preparation of the pollutant-spiked (oxy)hydroxides has to be carefully designed in order to represent a suitable model of the natural system being studied[17].

The column was conceived to allow sampling at different levels, thus giving access to profiles of physio-chemical and biological parameters. Thus, the system can include several layers that simulate *in situ* heterogeneities. Here, heterogeneities of iron concentration in natural aquifers were simulated; however, other types of mineralogical heterogeneities may be studied by adapting the type of synthetic mineral included in the silica gel. The silica gel matrix efficiently prevented the movement of fine particles of iron (oxy)hydroxides. In the given examples, the columns were inoculated with natural microflora from ground-water[19], however, as the column and all the associated equipment can be sterilized, experiments with pure bacterial strains may be considered.

The limitations of the technique are linked to the size of the experimental device. The amount of liquid that can be sampled from each sampling port must be limited to 5 mL (maximum) because the sampling disrupts the systems' equilibrium. The magnitude of disruption will be related to the feeding flow-rate: for very low feeding flow rates, the disruption will be greater than for higher flow-rates. Thus, the low sampling volume limits the range of measurements and analyses that can be performed. The frequency of sampling in sampling ports should also be limited to let the column reach a new equilibrium between each profile sampling. For similar reasons, the sampling of solid material through the sampling ports must be limited to very small amounts. Another limitation of the technique is the difficulty of performing reproducible experiments in multiple columns, since in continuous feeding conditions it would be very hard to maintain identical conditions in parallel devices.

The present experimental column setup acquires data relating to phenomena occurring in aquifers that cannot be obtained using batch conditions. In the frame of a complete study, it is a significant complement to classical batch experiments that can be performed in replicates[17,20].

Potential applications of this experimental setup include the elucidation of biogeochemical processes inducing the release of toxic TEs (*e.g.*, As, Se) from natural geological formations and the evaluation of the impact of anthropogenic activities on these processes, such as an input of nitrate or pesticides in groundwater, for example, or fluctuations of the groundwater level. The columns may also be useful in testing bioremediation options[31] for the biodegradation of organic pollutants or the stabilization of inorganic contaminants such as Hg.

Disclosures

The authors have nothing to disclose.

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