Video Article

Sediment Core Sectioning and Extraction of Pore Waters under Anoxic Conditions

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Abstract

We demonstrate a method for sectioning sediment cores and extracting pore waters while maintaining oxygen-free conditions. A simple, inexpensive system is built and can be transported to a temporary work space close to field sampling site(s) to facilitate rapid analysis. Cores are extruded into a portable glove bag, where they are sectioned and each 1-3 cm thick section (depending on core diameter) is sealed into 50 ml centrifuge tubes. Pore waters are separated with centrifugation outside of the glove bag and then returned to the glove bag for separation from the sediment. These extracted pore water samples can be analyzed immediately. Immediate analyses of redox sensitive species, such as sulfide, iron speciation, and arsenic speciation indicate that oxidation of pore waters is minimal; some samples show approximately 100% of the reduced species, *e.g.* 100% Fe(II) with no detectable Fe(III). Both sediment and pore water samples can be preserved to maintain chemical species for further analysis upon return to the laboratory.

Video Link

The video component of this article can be found at https://www.jove.com/video/53393/

Introduction

Researchers often wish to study the redox state and geomicrobiology of a sediment-water system. This ideally utilizes data from both sediments and pore waters, as pore waters are often sensitive monitors of the system and are a common source, though not the only source, of ecological exposure to redox-sensitive heavy metals[1] such as arsenic and uranium. Pore water data may be obtained *in situ* using diffusion equilibrium filters, also known as "peepers," installed into the sediment[2]. Peepers are most commonly used in settings where the field site is known prior to beginning field work and where multiple visits over an extended period of time can be made to the field site, *e.g.* Shotyk[3]. Therefore many contexts do not permit the use of peepers, such as sites only accessible for a short time or where multiple exploratory samples are obtained to determine where further investigation should occur[4]. Additionally peepers do not sample sediment simultaneously to water sampling.

When it is desirable to sample sediment and water together, or in field sites where peeper installation is not feasible, the most common method to obtain sediment and water is sediment coring. Obtaining an unmixed core is a crucial precursor to the procedure described in this work[5]. Once a core is obtained pore waters may be obtained by squeezing[6] or centrifugation; both have advantages and disadvantages. Centrifugation is generally considered the most reliable method for extracting porewaters from sediment cores,[7] although care must be taken to prevent oxidation of sediments or pore waters.

In this method we describe core extrusion and centrifugation to extract pore waters with minimal oxidation. The authors have used the method described herein in a variety of contexts including marine[8], contaminated lake[9], and wetlands[10]. The representative data shown demonstrates that reducing conditions can be preserved. With the exception of the centrifuge, materials used are inexpensive, and this method can be applied to a wide variety of geochemical and geomicrobiological research questions.

Protocol

1. Preparation of Equipment

1. Preparation of Core Liners
    1. Calculate thickness of core slice that will be obtained using $Volume = \pi r^2 \times thickness$; the final volume must be <50 cm^3. With a 10 cm core diameter, 2 cm thick slices may be obtained.
    NOTE: It is not necessary to have the volume be a full 50 ml, but porewater volumes obtained will be proportionately smaller.

2. Using a jigsaw (or similar) slice one core liner, or a plastic tube of identical diameter, into 2 cm rings (or other thickness if using a different diameter core). Obtain 3-5 rings.
3. Clean all plastic material that will come in contact with sediment, including core liners, core caps, rings, core slicers, centrifuge tubes, syringes, and disposable spoons. (Soak plastic materials in 10% HCl for 24 hr, rinse plastic materials 3x in nanopure (22 MΩ) water, and allow materials to air dry, preferably in a laminar flow hood, before packing.)

2. Preparation of Laboratory Jack
   1. Cut a piece of plywood, using a jigsaw, to cover the 6" x 6" size of the top plate of the laboratory jack and drill a hole in the center of this plywood using a hole saw attachment to a drill.
      NOTE: This hole should be just slightly larger than the diameter of PVC extender pieces; for the sizes used here, the hole should be 2 ¾".
   2. Drill four small holes in the edges of this plywood to match the holes on the top plate of the laboratory jack using a regular drill bit on a hand drill or drill press. Fasten the plywood to the laboratory jack with zip ties.
      NOTE: There should be no "wiggle" in this plywood.

3. Drill a hole in the center of an approximately 2' x 1.5' plywood slightly larger than the outer diameter of the core liners using a hole saw. For 10 cm diameter core liners, a 10.5-11.5 cm hole will be appropriate. This wood is the core guidance plate.
4. Preparation of Core Extruder (Plunger)
   1. Obtain a rubber laboratory stopper that fits snugly inside the core liners. If none is available, shave one to size using a razor. Do not use a too-small stopper.
   2. Screw the laboratory stopper to an approximately 1'-1.5' long, 1" diameter dowel or broom handle. Cover the screw head on the face of the stopper with waterproof electrical tape.
      NOTE: The larger side of the stopper should face away from the dowel.

5. Cut an approximately 1.5' length of PVC pipe into ~6" long sections.
   NOTE: The internal diameter should be larger than the dowel in step 1.4.2, but smaller than the stopper in step 1.4.1. This protocol assumes using standard 2" PVC with a true outer diameter of 2.375". These PVC pieces are the core extenders.
6. Assemble all other needed equipment listed in the Materials List.
   NOTE: This should be gathered in the home laboratory and brought to the field laboratory.

2. Setting up the Field Laboratory Station

1. Setting up the Glove Bag
   1. Clamp the core guidance plate to the work surface (countertop, lab surface, *etc.*). Ensure that the hole for the core liner is over the work surface, but is open.
   2. Place the disposable glove bag over the core guidance plate, and run the tubing from the tubing entry of the glove bag to the regulator of the N_2 tank. Make sure the tank is safely secured using the cylinder bench clamp.
   3. Tape the tubing to the glove bag to seal up this entry point by encircling the bag around the exterior of the tubing with electrical tape. Ensure that about 8" of tubing extends into the interior of the glove bag. Slide the tubing clamp onto the tubing inside the glove bag; leave this clamp open.
   4. Cut an "X" in the bottom of the glove bag over the hole in the core guidance plate using a box cutter or straight razor. This x should be smaller than the core liner diameter.
   5. Load the glove bag with the items found in **Table 1**.

2. Setting up the Core
   1. Place the laboratory jack on the floor underneath the work area where the glove bag is affixed. Place the core through the "X" cut in the bottom of the glove bag in step 2.1.4, keeping it in an upright position.
      NOTE: About 4-6" of the core should extend above the core stabilization plate.
   2. Hold the core steady to allow researcher 2 to perform steps 2.2.3 through 2.2.6.
   3. Tape the core to the plastic glove bag around the "X", using plenty of good sealing electrical tape.
   4. Insert the handle of the core extruder into a 2" diameter, ~6" long PVC spacer, followed by a ~3" long coupling, followed by another spacer. Continue this pattern until the PVC entirely covers the handle; it may extend a short distance past the end of the handle. Place the core extruder (with PVC spacers and couplings) underneath the bottom of the core.
   5. Support the core extruder with the laboratory jack so that the extruder can support the core. At this time, keep the jack as low as possible and use core spacers wherever possible.
   6. Use a box cutter to cut carefully around the bottom core cap. This cut should result in a ring of core cap being left around the outside of the core liner and a flat circular portion of the core cap in place against the core materials.
   7. Insert the hands into the glove bag (researcher 1). Hold the core steady so that it does not move further above the core stabilization plate while researcher 2 begins to turn the laboratory jack slowly to raise the core. The core extruder should enter the core liner and begin to push the sediment upwards like a push pop.
   8. Wiggle the core extruder side to side slightly if needed to insert it into the core liner (researcher 2). Be prepared for a slight pop when it enters.
   9. Continue to raise the core until the top of the cored material is at or near the top of the core liner (researchers 1 and 2). Note that the core cap should remain atop the core during this procedure.

3. Sealing the Glove Bag
   1. Double check that all necessary supplies are in the glove bag; turn on the portable oxygen meter.
   2. Open all centrifuge tubes, water bottles, and other items that contain trapped air. If the core has headspace above the standing water, open the top cap to purge this headspace.

3. Turn on the regulator so that the flow of nitrogen through the tube into the glove bag is at a moderate rate. Generally a pressure of ~15 psi at the last regulator stage with all valves open is appropriate.
   NOTE: This should be fast enough to feel like a strong breeze on skin, but not so strong that it scatters glove bag supplies.
4. Point the nitrogen into all areas of the glove bag, and push the nitrogen out the main opening to the best of one's ability. Turn off the nitrogen.
5. Purge the glove bag three times by repeating steps 2.3.6 through 2.3.9 three times.
   NOTE: One person can accomplish this, but it may be easier for two people so that one can keep his or her hands in the gloves that are a part of the glove bag.
6. Seal the main opening of the glove bag by wrapping 1-2 bungee cords around it several times. Turn on the nitrogen to a similar flow as in 2.3.3. Place researcher 1's hands in the built-in gloves of the glove bag.
7. Move the nitrogen tube from one area of the bag to another to fill the glove bag with nitrogen. Point the tube into any crevices such as opened centrifuge tube, above the water in a squirt bottle, *etc.*
8. Turn off the nitrogen by closing the tubing clamp. Note that this can be accomplished without removing researcher 1's hands from the glove bag.
9. Open the front of the glove bag and using researcher 1's body, push as much gas out of the bag as possible.
   NOTE: The bag should be flat around the supplies inside it at this point.
10. Fill the glove bag to a comfortable pressure and turn off the nitrogen using the tubing clamp. Some trial and error may be required to find a comfortable pressure, as too full and it is difficult to move one's arms, while too empty and it is difficult to see and manipulate objects. Open the nitrogen tubing clamp for short periods to refill the bag if it seems to have a slow leak or is getting emptier for any reason. Open the front of the bag to let out small amounts of nitrogen if it becomes uncomfortably full.
11. Check the level of dissolved oxygen on the portable oxygen meter. It should be below 1%.
12. Insert the glove bag gloves into disposable gloves. These will improve dexterity. Change them whenever they get dirty or torn; discard in the in-bag waste container.

3. Sectioning the Core

NOTE: this portion of the procedure is much more easily accomplished with two researchers.

1. Remove standing water using a syringe. Syringe filter this water and place into 50 ml centrifuge tubes.
2. Remove a Core Section
   1. Place a core sectioning ring above the top of the core liner. Raise the core up (section 3.3) until the top of the sediment is at the top of the ring.
   2. Insert the core slicer between the top of the core liner and the ring. The sediment section is now sitting atop the core slicer.
   3. Move the sediment to a 50 ml tube using the disposable spoons. Cap the tube tightly once it is full.
   4. Rinse the core slicer and core sectioning ring with the nanopure water; squirt the dirty waste into the waste container inside the glove bag. Disposable spoons can also be rinsed, or discarded, depending on the number needed. Dry the core slicer and core sectioning ring with paper towels.
   5. Repeat step 3.2 until no further core material remains.
3. Raising the Core
   1. Throughout 3.2 it is necessary to raise the core. Do this in small increments using the laboratory jack. Perform the following three steps once the laboratory jack is fully extended.
   2. Hold the core in place in the glove bag (researcher 1).
   3. Lower the laboratory jack to its lowest setting, when it is fully compressed (researcher 2). Ensure that the core is held in place with researcher 1's hands within the glove bag and researcher 2's hands supporting from beneath.
   4. Fill the space between the bottom end of the core extruder and the jack with PVC spacers and fittings. Ensure that the core is securely supported by the core extender before letting go of the top of the core.

4. Extracting Porewaters

1. Open the glove bag and remove the rack of centrifuge tubes.
2. Clean up the glove bag as needed by removing any soil, liquid, or condensation by wiping out the inside with paper towels, water, *etc.* Empty waste container.
3. Reseal the glove bag loosely with bungee cords.
4. Weigh centrifuge tubes which now contain sectioned sediment (if desired).
5. Wrap the tops of the centrifuge tubes with electrical tape to seal the cap/tube junction.
6. Balance the tubes for centrifugation by weighing them after taping. Add or remove electrical tape to get weights within 0.5 g.
   NOTE: Generally, tubes from the top of the core will be lighter than those from the bottom.
7. Place tubes in centrifuge and centrifuge at maximum acceleration (1,100 x g is recommended) for 20 min.
   NOTE: Higher centrifugation rates will permit greater separation of porewaters.
8. Remove electrical tape from the centrifuge tubes either by unpeeling or by slicing with a razor prior to returning the tubes to the glove bag. Return centrifuge tubes to rack after centrifugation and return to the glove bag.
9. Add another set of centrifuge tubes, syringes, and syringe filters to the glove bag. These new tubes will hold porewaters and can be pre-labeled.
10. Purge the glove bag again as in step 2.3.5. If the glove bag was only opened briefly to remove the tubes, and was not cleaned, it will be acceptable to purge it only two times. If an oxygen monitor is being used, ensure that the atmosphere in the glove bag is <1% O_2. Do not forget to open the newly added centrifuge tubes and purge them.

11. Insert hands to the gloves in the glove bag; as before, cover with disposable gloves.
12. Open one tube. Remove the porewaters from above the sediment using a syringe, then attach a syringe filter to the tip of the syringe. If porewaters and sediments are well separated, porewaters may be poured directly into a syringe with the barrel removed and a syringe filter on the tip.
13. Push the water through the syringe filter into the appropriate centrifuge tube.
    NOTE: Some force will be needed; more than one syringe filter per sample may be required.
14. Replace the caps on the centrifuge tube containing sediment and the tube containing porewaters.
15. Repeat steps 4.13 through 4.15 for each sample.
16. Open glove bag and remove samples. Both sediment and porewater tubes can be re-weighed.
17. Analyze porewaters immediately if desired. Analyses can include (but are not limited to) ion chromatography for major ions[11], ferrozine for iron speciation[12], arsenic speciation by voltammetry[13], and sulfide speciation[14].
18. Freeze porewaters and sediments in the dry shipper (approximately -80 °C) to preserve speciation for later analyses if appropriate for the planned analyses. It may also be appropriate to keep samples cool in airtight containers or in nitrogen purged aluminum bags.
19. Re-use the glove bag for a second core (starting at 2.2.2) if desired. After two cores, the glove bag usually needs to be changed.

Representative Results

The type of results obtained depends on analyses performed and on the geochemical setting from which the core was obtained. Dissolved oxygen may be measured in the extracted porewaters, but in many settings this will be zero below the first few cm of the core. Analyses that usually provide more meaningful information include iron speciation (Fe II/Fe III)[12], arsenic speciation (As III/As V)[13], and sulfide[14]. Presence of reduced species such as sulfide indicates both a reducing environment and that sufficient anoxia was maintained during core sectioning and pore water removal. Determination of other concentrations such as dissolved organic carbon, major ions, or trace metals is often performed on preserved samples upon return to the home laboratory. Geochemical gradients can generally be observed in pore waters, and maxima or minima of particular species may be seen at depth.

A core was taken in Bay Batiste, a wetland southeast of New Orleans, about nine months after the beginning of the Deepwater Horizon spill. This wetland was heavily oiled, and data obtained from the sediment core indicate high sulfide concentrations in the porewaters using a Hach Method (http://hach.com) based on[14]; see **Figure 1**. Maximum sulfide concentrations of 49.2 mg/L S^{2-} are observed in the core section obtained between 24-27 cm depth. Total iron concentrations in these porewaters were consistently low (<0.2 ppm) and no Fe(III) was detected.

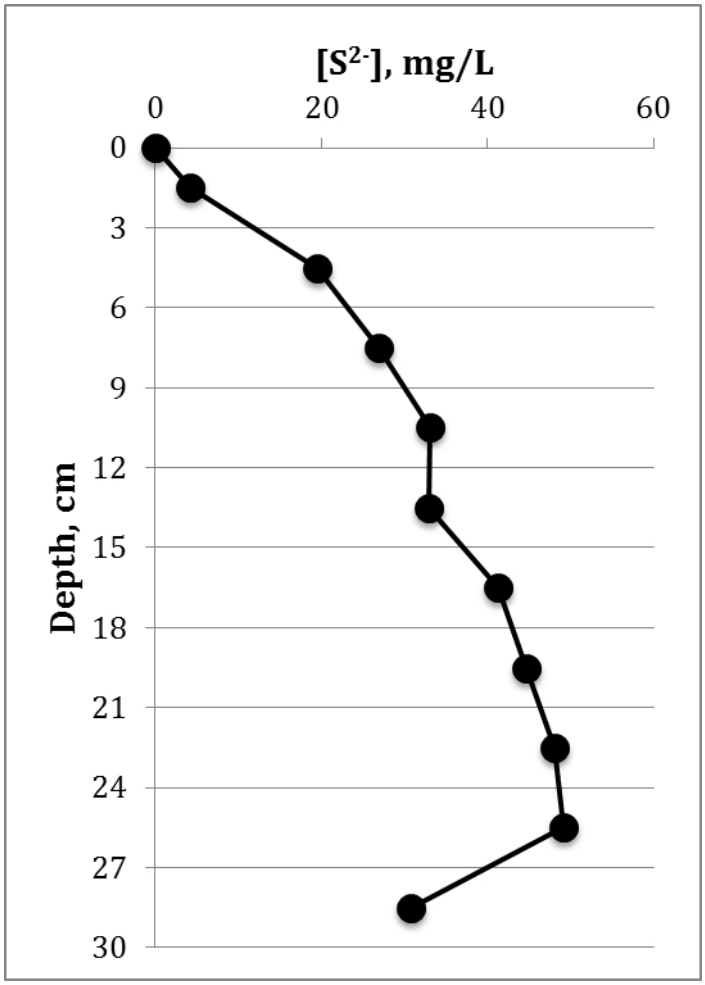

Figure 1: Porewaters from Bay Batiste, Louisiana. Data shown is from porewaters extracted from a sediment core using the methods described herein; the core was obtained from Bayou Batiste, Louisiana, in the year following the Deepwater Horizon oil spill to the Gulf of Mexico. Dissolved sulfide concentrations in porewaters as a function of depth below the sediment-water interface. Please click here to view a larger version of this figure.

Items to be loaded into glove bag at Step 2.1.7
Waste containers
Box of disposable gloves
Kimwipes and paper towels
Straight razors
Squirt bottle(s) of dd H_2O
Permanent marker
Disposable plastic spoons
50 ml centrifuge tubes[a] in a rack; one per core section plus enough for overlying water.
Syringe filters sufficient in number for filtering overlying water.
Core liner rings
Core slicers
Portable Oxygen Meter
[a] Plastic materials such as centrifuge tubes should be acid-cleaned according to instructions.

Table 1: Materials to seal into glove bag.

Discussion

The technique described herein is a flexible one that can be adjusted for a wide range of locations, core sizes, core section thickness, *etc.* There are three essential components to this system.

First, prepare a core extrusion system of the right dimensions for the core to be analyzed. Instructions here are given assuming an approximately 30" core; much longer cores may require more PVC extender pieces and PVC fittings to extrude fully. Plan the extrusion system and the packing carefully, as corrections in the field are much more difficult to manage.

Second, ensure that the glove bag is well purged and free of leaks or tears. The purpose of this protocol is to obtain porewaters in the same redox state in which they existed below ground. If oxidation occurs during pore sectioning or pore water extraction, the data obtained will not be usable.

Third, the centrifugation immediately allows separation of the porewaters from the sediments. If porewaters and sediments remain in contact after removal from the environment, reactions may continue and change. For example, if water flowing through the core was supplying nitrate, this would prevent the existing microbial community from using iron as a terminal electron acceptor; after removal of the core from the site, nitrate concentrations would begin to decrease and iron speciation could begin to change. Therefore rapid core sectioning and centrifugation allow the best "snapshot" to be taken.

Depending on the analyses desired, it may be desirable to weigh the centrifuge tubes before filling them with sediment and porewater. This will allow computation of the exact masses of porewater and sediment collected from each section. If this is not necessary, an average mass of the 50 ml centrifuge tubes may be assumed for each tube. This is usually sufficient. In general, a subsection of sediment may be removed, weighed, and dried again to obtain a percent dry mass value. When doing this, make sure to include the weight of porewaters removed as part of the calculation. Dried sediment may also be combusted to obtain a loss on ignition measurement.

It may be advisable to practice this procedure once or twice on a sample core before performing it on valuable field samples. Once it is mastered, however, this technique allows collection of pore waters and sediments from a wide range of environments in a straightforward, cost-effective way. The ability to maintain the *in situ* redox conditions allows a range of geochemical and geomicrobiological analyses on the collected samples.

Disclosures

The authors have nothing to disclose.

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