ASSESSMENT OF THE EFFECTS OF BIOTURBATION IN CONTAMINATED SEDIMENTS

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ABSTRACT

Oligochaete worms are often the dominant organisms present in contaminated sediment in the field. Bioturbation, the reworking of sediments or soils by organisms, can be a significant fate and transport mechanism in contaminated sediment systems. The fate of pyrene, a polycyclic aromatic hydrocarbon, subjected to oligochaete bioturbation is the focus of this paper. *Limnodrilus cervix,* a representative oligochaete, were introduced into small laboratory microcosms containing beds of contaminated sediment with a continuous flow of overlying water. Through their feeding, respiration, and burrowing activities, the worms physically changed the porosity of the sediment and significantly influenced the fate and transport of pyrene by several mechanisms. Over a seven-month experiment, the amount of pyrene released into the overlying water was about 250% greater in bioturbated than in undisturbed cells. Pyrene loss in the upper 15 mm due to biotic processes was 170% greater than undisturbed cells due to enhanced microbial processes and/or direct worm degradation. The pyrene remaining in the upper 15 mm of sediment bed at the end of the experiment was 15% for bioturbated cells compared to 52% for undisturbed controls. Experimental results suggest that native oligochaetes can enhance natural attenuation of contaminated sediments in the field.

Key words: *oligochaete bioturbation, PAH, sediment, natural attenuation*

INTRODUCTION

Sediments serve as sinks for hydrophobic organic contaminants in the environment. To assess contaminated field sites, an understanding of different possible fate and transport mechanisms is needed. In high energy systems, turbulent water scours the sediment surface and erosion and deposition mechanisms significantly impact the movement of hydrophobic contaminants. In low energy systems, however, bioturbation–the reworking of sediment or soil by living organisms–can be the dominant mechanism.

In a historically contaminated benthic system, typically several of the more sensitive forms of life have been eliminated. Without competition, the most tolerant organisms survive to proliferate to large populations. In highly contaminated field sediments, tubificid oligochaetes are frequently observed to be the dominant lifeform. Tubificids are small burrowing worms often about 2-5 cm long and roughly 1 mm in diameter when fully mature. Body weights of mature tubificids vary widely but are usually 1 mg (dry) or less. The bioturbation activities of tubificid worms consist of feeding, breathing, and burrowing.

Tubificids typically feed by placing their heads down into the sediment and leaving their tails protruding into the overlying water. Particles of sediment ingested at depth then pass through the worm and are egested onto the sediment surface in small mounds of fecal pellets. The feeding rate of oligochaetes depends heavily on temperature conditions. Appleby and Brinkhurst (1970) reported that *Limnodrilus hoffmeisteri,* a common and widespread tubificid oligochaete, processed an average of 44 times their dry weight per day at 21.7°C. While a substantial amount of feeding occurs with defecation at the sediment surface, the worms can also remain totally submerged during feeding. Depending on benthic conditions, the depth of feeding can be shallow or extend to depths of 10 cm. Through their feeding behavior, oligochaetes thoroughly mix sediment particles in the upper bioturbated zone.

The respiration of benthic worms can have a profound effect on the exchange of water between the sediment bed and the overlying water. The worms use the dissolved oxygen present in the ingested water to breathe. *Limnodrilus hoffmeisteri* has been reported to consume about 6.8 mL of oxygen per worm per day (Beck, 1972). The worms can later release the water after they have completely submerged back into the sediment bed. In a mixed culture of *Limnodrilus hoffmeisteri* and *Potamothrix vejdovskyi,* 9.5 to 15.0 mL of water per worm per hour was "pumped" into the sediment by respiration activity (Wood, 1975). The breathing mechanism of the worms effectively brings about an enhanced rate of pore water exchange with the overlying water and increases the amount of oxygen present in the sediment bed.

The burrowing of oligochaete worms also substantially impacts the sediment. The depth of burrowing for a given population of bioturbators differs according to a variety of benthic conditions but typically extends deeper than the zone of feeding. Oligochaete burrows appear as thin, cross-linked gaps in the sediment depth profile. Older burrows eventually consolidate to be replaced by fresh burrows. The burrowing of oligochaetes has been reported to increase the moisture content in the bioturbated zone of the sediment bed (Tevesz *et al.*, 1980).

The focus of this study is to assess how oligochaete bioturbation–feeding, respiration, and burrowing–impacts the fate and transport of polycyclic aromatic hydrocarbons (PAHs). Produced by natural and anthroprogenic sources, PAHs are ubiquitous environmental contaminants that due to their hydrophobicity frequently end up in soils and sediments. PAHs are of significant environmental concern due to some being either confirmed and/or suspected carcinogens and mutagens (Harvey, 1991).

Several different fate and transport processes are influenced by oligochaete bioturbation. Flux, the release of contaminants from the sediment bed into the overlying water, is significantly higher in bioturbated sediments compared to undisturbed sediments (Reible et al., 1996). Biotic degradation can also be a key mechanism. Some benthic organisms have demonstrated the ability to directly biotransform PAHs and bioturbation can also increase oxygen transport into sediment, which may potentially enhance aerobic microbial processes. These mechanisms will be explored in this paper.

Another fate and transport mechanism is the progression of PAHs up the food chain. PAHs are classified as being bioaccumulative contaminants of concern (Bridges et al., 1996). Contaminants can be ingested or absorbed dermally by benthic organisms and subsequently partition onto the lipid material. Because oligochaetes are directly exposed to contaminated sediments and are at the bottom of the food chain, they would be a significant organism to use in bioaccumulation studies. A related study of pyrene bioaccumation by *Limnodrilus hoffmeisteri* was presented at this conference (Millward et al., 1999).

The objective of this paper is to present the results of a seven-month experiment detailing the effects of oligochaete bioturbation on PAH-contaminated sediments. Using a longer time frame allowed the measurement of small changes that would be difficult to evaluate during a short-term experiment. A lengthy experiment was also desirable so that the different fate and transport mechanisms could be assessed in terms of which processes are most significant over a sustained period of time.

MATERIALS AND METHODS

Pyrene, a representative four-ring PAH, was selected for this experiment. Pyrene is a strongly hydrophobic compound that is only slightly soluble in water (maximum solubility ~140 ppb) and has a log Koc value of about 5 (Montgomery, 1996).

Sediment was collected from Campus Lake, a freshwater lake on the Louisiana State University campus in Baton Rouge, La. The Campus Lake sediment was characterized (A&L Analytical Labs, Memphis, Tenn.) to be a silty loam with an organic carbon content of 1.12%. After collection, the sediment was wet-sieved through a 2 mm (No. 10) mesh and then frozen and thawed twice to eliminate native macrolife. No attempt was made to eradicate native bacteria or add other bacteria to the sediment.

Freshwater tubificid oligochaetes, identified as the genus *Limnodrilus* and consisting predominantly of *Limnodrilus cervix*, were located at a field site in Baton Rouge, La. The *Limnodrilus* genus is widely distributed and frequently found at contaminated sediment sites. For the experiment, an initial population density of about 26700 worms per square meter was selected. This is a moderate number of what is observed in the field. Oligochaete populations have been reported at densities greater than 1,000,000 worms per square meter (Palmer, 1968).

The clean Campus Lake sediment was inoculated in the laboratory with pyrene (99% purity, Aldrich Chemical Company, Inc., Milwaukee, Wisc). To spike the sediment, pyrene was plated onto the inner walls of a glass jar and a sediment slurry was added. The glass jar was then sealed and mixed on an axial tumbler for one month to ensure homogeneity. The desired loading was targeted to be ~60 mg/kg dry (ppm), the IC 25 level for *Limnodrilus* worms. IC 25 is an environmental endpoint where there is a 25% reduction in reproduction and sediment ingestion rates (Lotufo and Fleeger, 1996). The resulting loading was confirmed to be 64 mg/kg dry (ppm). After inoculation, the sediment was stored in the dark and "aged" for over 100 days before the commencement of the experiment.

The overlying water consisted of aerated tap water with no food or nutrients added. Attempts were made to keep the overlying water temperature (21.5°C ± 1.5) and dissolved oxygen concentration (4.08 mg/L ± 0.74) constant. These values are in the range where oligochaetes will be most productive (Mason, 1994).

The experiment took place in two multi-cell Plexiglas chambers previously used by our laboratory (Reible et al., 1996). These chambers contain independent cells separated by 0.64 cm walls. Each cell is 15 cm long, 5 cm wide and holds a 3.5 cm deep sediment bed. A schematic of the experimental setup is pictured in Figure 1. The flow of water from the source tanks was controlled by a multi-channel peristaltic pump which maintained a flow rate of 300 mL/hr for each cell. The experimental chambers rested in a 30°C water bath held at constant temperature by a recirculating heater. A thin layer of water passed in a continuous laminar flow over the sediment bed. When the effluent water from each cell was not being sampled, all the exiting water accumulated in a collection drum before being cleaned by an activated carbon filter.

The experimental chambers were covered by glass plates and carefully sealed with vacuum grease. Previous work in our laboratory has verified that any volatization losses of pyrene from the closed experimental system under these conditions would be at most negligible. In addition, the microcosms were covered by aluminum foil. While the purpose was to keep the worms in darkness, this also indirectly prevented the photodegradation of pyrene on the sediment surface.

Pyrene concentrations in sediment and aqueous samples were collected over the course of the experiment. Sediment samples were analyzed by a solid-liquid extraction procedure based on EPA SW-846 method 3550A. Samples of effluent water leaving the microcosms were analyzed by liquid-liquid extraction. In each case, sample extracts were analyzed by a HPLC (HP 1090) using UV and/or fluorescence (HP 1046A) detection.

RESULTS & DISCUSSION

The experiment began with five worm and three control cells. One worm and one control cell were sacrificed in the interim to assess the progress of the experiment. The data presented in this paper are the average of the four worm and two control cells that completed the entire seven-month experiment.

Contaminant flux was monitored over the course of the experiment. The amount of pyrene released into the overlying water was measured by collecting an effluent sample from each cell periodically over the course of the experiment. In general, the flux from the bioturbated cells was significantly higher than from the undisturbed cells. Flux was greatest in the early months of the experiment but gradually decreased. To calculate the total amount of pyrene released, the concentrations measured over time were interpolated between sampling periods. Over the course of the seven-month experiment, flux was 250% greater in the worm cells than in the undisturbed control cells.

The sediment-water interface is sometimes poorly defined in the field. In controlled laboratory conditions, however, it was possible to carefully core the sediment bed in 1 mm slices to a depth of 15 mm at the conclusion of the experiment. A portion of each slice was analyzed for moisture content by drying in a 105°C oven for 24 hours. The impact of oligochaete bioturbation on sediment beds is clearly shown in Figure 2. The dashed line signifies the initial moisture content of the beds when they were carefully loaded at the beginning of the experiment. The undisturbed cells are unchanged at 2 mm or deeper, but in the worm cells the effects of burrowing and respiration are clearly observed to a depth of about 14 mm.

A profile of contaminant concentration was obtained by analyzing a portion of each 1 mm core sample for pyrene concentration. The profile of pyrene concentration as a function of depth for worm and control cells is shown in Figure 3. The dashed line represents the initial loading of 64 mg/kg dry. The upper 2 mm of the control cells are completely depleted, followed by a gradual increase until about 9 mm where pyrene concentrations level off. The worm cells, on the other hand, are heavily depleted throughout the profile. The loss of pyrene is attributable to release and degradation.

In order to distinguish the significance of the different fate and transport processes at work, a mass balance for pyrene was constructed around the upper 15 mm of each cell. Since the total mass of dry sediment from the upper 15 mm is known for each cell and the initial loading was measured at the beginning of the experiment, the mass of pyrene initially present in the cored material was calculated. Likewise, the amount of pyrene remaining in each 1 mm section of the profile for each cell was determined. The average pyrene mass balance for the two control and four worm cells is summarized in Figure 4.

The flux of pyrene from the bioturbated sediment accounted for a loss of about 13% of the initial pyrene in the upper 15 mm of sediment, two and a half times more than the undisturbed sediment. Although individual release mechanisms were not studied, the increased flux of pyrene is attributed to the feeding, respiration, and burrowing activities of the worms. Through their feeding and burrowing, the worms mixed the sediment and continually replenished the pyrene at the surface. Oligochaete respiration enhanced the exchange of pore water; since contaminants partition to pore water, the water exchange displaced the existing interstitial water into the overlying stream and enhanced the release of contaminants. By loosening the upper part of the sediment bed, the diffusion of pyrene into the overlying water was also increased. Flux was highest in the early months but dropped off towards the end of the seven-month experiment. The decrease in flux over time is attributed to depletion of pyrene in the upper sediment layer.

The most significant part of the mass balance, however, is the "missing" pyrene from both the worm and control cells. Since abiotic degradation or volatilization losses from system are minimal (see explanation in Material and Methods section), the "missing" pyrene was presumed lost due to biotic processes. No attempt was made to search for possible metabolites in this experiment. In both the worm and control cells, biotic loss was a much more significant mechanism than flux. Further, biotic loss in the bioturbated cells was 170% greater than that of the undisturbed cells. Possible mechanisms for biotic degradation include biotransformation by *Limnodrilus cervix* and degradation by aerobic bacteria. Unfortunately, results do not provide quantitative insight into which of the processes might be more significant.

Since biotransformation of PAHs by benthic organisms has been reported in the literature, direct biotransformation of pyrene by *Limnodrilus cervix* is a reasonable explanation of the loss of pyrene from the system. Preliminary work measuring pyrene depletion in organism fecal matter tentatively suggests that *Limnodrilus hoffmeisteri* can directly biotransform PAHs (Shephard, 1997; Millward *et al.*, 1998). Future experiments are planned to confirm and quantify pyrene biotransformation by *Limnodrilus hoffmeisteri*.

The existence of PAH-degrading bacteria, particularly for PAHs with four or fewer rings, have been reported numerous times in the literature. No attempt was made to isolate and identify the bacteria present in Campus Lake. One study indicates that bacteria with the ability to degrade PAHs are indigenous to geographically diverse soils (Mueller et al., 1994). If clean sediment is subsequently polluted, given favorable conditions, this bacteria will demonstrate the ability to degrade the PAHs.

The worms may have facilitated an increase in bacteria activity over what occurred in the undisturbed cells. This scenario is supported by the changes to the sediment brought about by oligochaete burrowing and respiration. Because worm respiration irrigates the sediment, the amount of dissolved oxygen present in the porewater of the worm cells was continually replenished faster than that of the control cells. An increased oxygen supply indicates that additional aerobic bacteria activity would be possible. Also, the burrowing of the worms could loosen the sediment bed and allow better diffusion of oxygen deeper into the bed. Although it is uncertain which biotic loss mechanism was the most significant process, it is clear that oligochaete bioturbation increased the depletion of pyrene almost twofold.

CONCLUSIONS

Oligochaete bioturbation had a drastic impact on both the sediment and the pyrene over the course of a seven-month experiment. Changes to the sediment included an increase in the porosity and moisture content in the upper 14 mm of the bed. Release of pyrene was measured to be 250% greater in the bioturbated cells compared to the undisturbed cells. The most significant fate and transport mechanism was determined to be biotic loss processes, although this experiment did not distinguish between possible loss of pyrene by metabolic processes of the worms or by native bacteria enhanced by the activity of the worms.

The results from this laboratory experiment indicate that biotic degradation is a key fate and transport mechanism in contaminated sediments subjected to oligochaete bioturbation. Further work is needed to distinguish between degradation by bacteria or *Limnodrilus* worms. The metabolic pathway(s) also need to be studied to determine if benign end products ultimately result. In assessing whether oligochaetes assist in the remediation of contaminated sediments, the potential benefits of degradation must be weighed against the hazards posed by the increased release into overlying water due to bioturbation or bioaccumulation and movement of contaminants up the food chain. Based on the current results, oligochaete bioturbation in contaminated sediments appears to be a positive step towards natural attenuation efforts.

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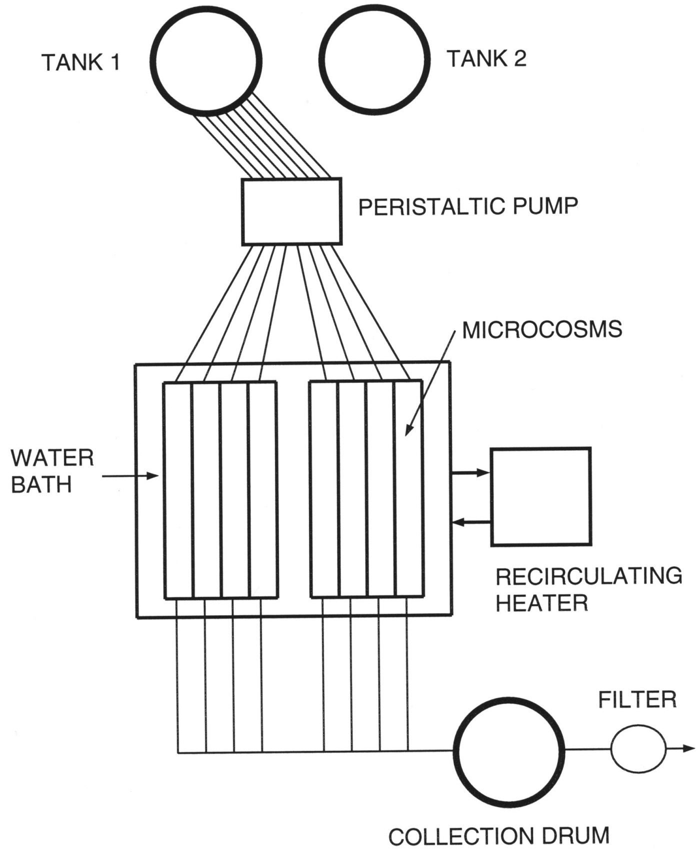

Figure 1. Schematic of experimental system.

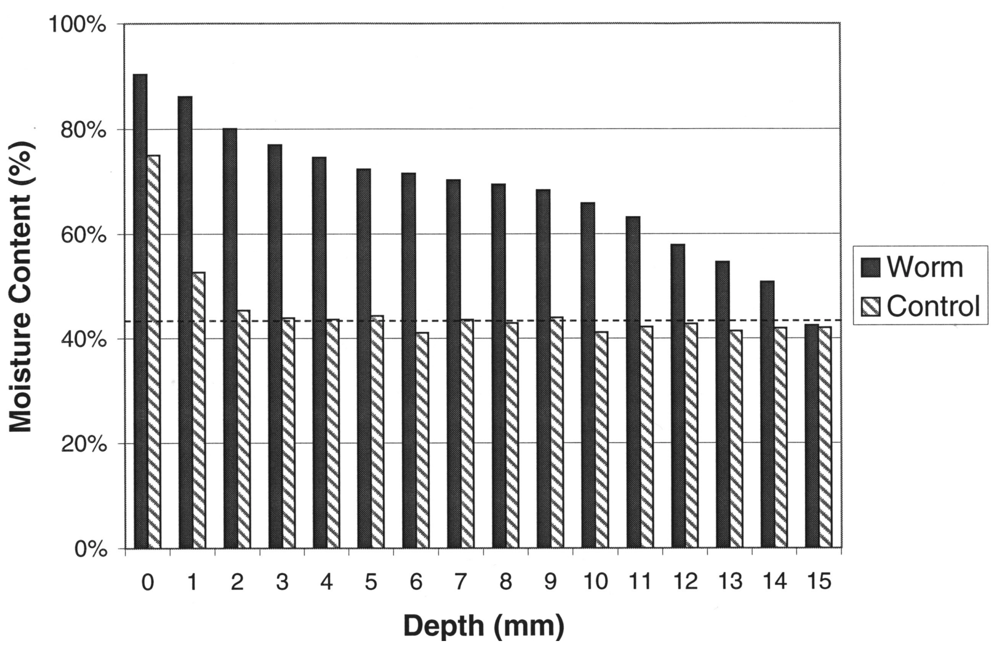

Figure 2. Profile of moisture content as a function of depth.

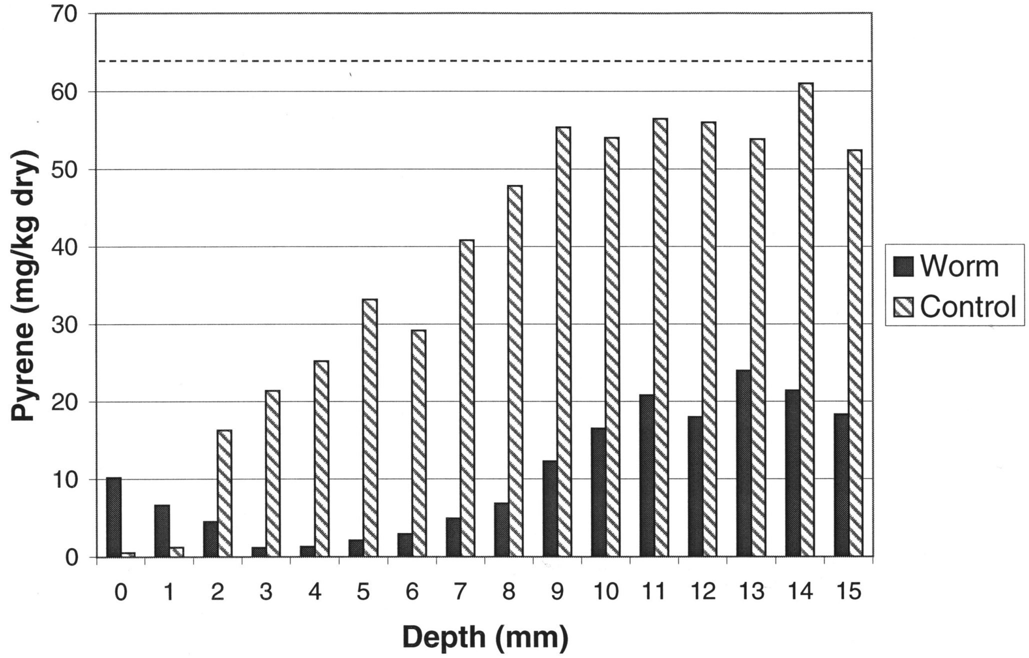

Figure 3. Profile of pyrene concentration as a function of depth.

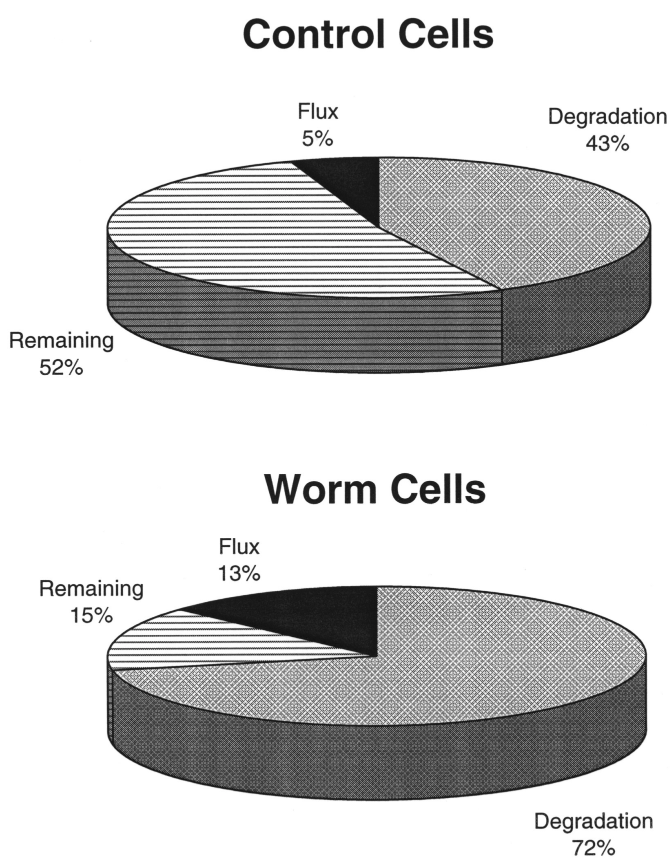

Figure 4. Pyrene mass balance.