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A LABORATORY MESOCOSM AS A TOOL TO STUDY PAH DEGRADATION IN A COASTAL MARSH WETLAND

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ABSTRACT 300206:

Polycyclic aromatic hydrocarbons (PAHs) are one of the contaminants of concern in coastal marsh environments which are subject to crude oil spills. A laboratory scale mesocosm can be used to complement field study of PAH degradation in coastal marshes. Coastal marsh wetland features, such as its soil, tidal cycles, and flushing, that may play roles in PAH degradation can be simulated in a laboratory mesocosm. The laboratory mesocosm tank is made of acrylic as the main construction material with an air chamber inside the tank which functions as a pneumatic system and tidal water storage compartment. Two trays filled with contaminated marsh wetland soil are situated at two different levels: the lower one is constantly submerged while the higher one is intermittently drained. When the air pressure inside the air chamber is high, the water will flow out from the air chamber to the tank to create high tide. When the air pressure inside the air chamber is low, the water will flow back from the tank to the air chamber to create low tide inside the tank. The tidal water sits in the air chamber until the next high air pressure. The cycles of air pressure inside the tank are controlled by an electrical air pump connected to a timer. The experimental setup can consist of several replicates with an air chamber inside each replicate is controlled by a master pneumatic tank. The model PAH contaminant used in the experiment was phenanthrene, a three-benzene-ring PAH, which was spiked to the wetland soil. The experimental results show that the phenanthrene degradation in the intertidal wetland soil is higher than that of in the subtidal wetland soil presumably due to the availability of oxygen in the intertidal wetland soil. The laboratory mesocosm developed in this study can be used as a tool for examining PAH degradation and other non-volatile organic contaminants.

INTRODUCTION:

Louisiana coastal marsh wetlands which support the largest commercial fishery in the lower 48 states are vulnerable to oil spills from the deep sea in the Gulf of Mexico. The oil spills have been swept into Louisiana salt marshes in coastal area zones by tidal movements and winds. Crude oils spilled at sea are immediately subject to a wide variety of weathering processes that affect their chemical compositions and physical properties (Kaplan *et al*., 1997; Wang *et al*., 1994; Bence *et al*., 1996; Short and Heintz, 1997; Wang *et al*., 1995, Wang *et al*., 1998, Page *et al*., 1996; Sauer *et al*., 1993). After several days of weathering processes, many of the spilled crude oil components can be expected to have relatively low concentrations when they reach the

Louisiana coastal wetland. The oil components that reach the coastal area are expected to continue to degrade physically, chemically, and biologically.

The accumulations of crude oils are damaging to coastal wetlands since oils are abundant with polycyclic aromatic hydrocarbons (Reilly, 1999). Polycyclic aromatic hydrocarbons (PAHs) are compounds built from two or more fused benzene rings or aromatic rings; they are also formed by incomplete combustion of carbon containing fuels. There are 18 PAHs considered by the Agency for Toxic Substances and Disease Registry (ATSDR) that have health effects; they are: acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*e*]pyrene, benzo[*b*]fluoranthene, benzo[*ghi*]perylene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, chrysene, coronene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, phenanthrene, and pyrene. They are toxic to a variety of marine species. PAHs in coastal environments tend to stay longer in sediment due to their hydrophobicity (low solubility and high octanol-water partition coefficient). Therefore, PAH concentrations are much higher in the sediments compared to those in the water column. Several physical, chemical, and biological processes degrade PAHs in soils, but biodegradation is probably the most effective process (Alexander, 1999 and Lu *et al*., 2011). Microorganisms utilize only dissolved substrates; and the utilization rates of PAH degradation products are related to sorption/ desorption rates of PAHs to/ from the soil.

Degradation of hydrophobic PAHs occurs mostly in the sediment water interface where they are adsorbed. Most coastal wetlands are exposed to tidal cycles: some wetland soil is subtidal (always submerged) and other type is intertidal (the soil is exposed to the atmosphere intermittently). Degradations of PAHs are expected to be different between the subtidal and the intertidal wetland soil. In-situ examination and laboratory experiments can be performed to understand the degradations and other pathways of PAHs. The in-situ or field study may be difficult to conduct because of limited control, while the laboratory experiment can be performed with some controls. Therefore, a laboratory mesocosm simulates the tidally influenced marsh wetland was designed and built to study PAH degradation. The mesocosm can simulate a certain environment and condition and may be reliable and cost effective for environmental fate test. Mesocosm experiments can be used to compliment field studies where many variables involved.

LABORATORY MESOCOSM DESIGN:

The laboratory mesocosm in this study was designed to study kinetic of degradation of non-volatile organic compound under tidal influence. The rectangular mesocosm is made of acrylic and has a total volume of about 31-L. It has an air tight chamber with an air flow hole, a lower tray of the subtidal wetland soil, a higher tray of the intertidal wetland soil, a dilution tank, and an overflow outlet hole (Figure 1). The air chamber is built inside the tank using acrylic with an upside down “u” shape hanging 5 mm from the bottom. The air chamber has a shorter length compared to the tank to create two gaps, which function as chutes for the water to flow in and out of the chamber. The air pressure inside the air chamber is controlled by an electrical air pump which is connected with a timer to set the time and the duration of the air pump activation and deactivation.

The tank is filled with about 12-L artificial sea salt water. The air chamber controls the water levels inside the tank. When the air pressure inside the air chamber is high, the water inside the air chamber is pushed out through the chute into the mesocosm tank. When the air pressure inside the air chamber is low, the water from the mesocosm tank flows back to the air chamber through the chute to replace the air. The water level inside the mesocosm tank is related to the air depth inside the air chamber (see Figure 2). A high air volume in the air chamber forces the water out raising the water level in the mesocosm. On the other hand, lowering the air depth in the air chamber lowers the water level in the mesocosm tank.

The wetland soil trays are also made of acrylic with the size of 15 cm x 20 cm x 5 cm (volume = 1.5 L). Wetland soil is placed inside the tray where water can flow vertically during water level changes without losing the soil. Two trays are placed above the air chamber: one is higher than the other. The lower tray represents the subtidal wetland soil, while the higher represents the intertidal wetland soil. The subtidal wetland soil is always flooded with water, while the intertidal wetland soil is covered with about 5 cm water above the soil during high tide. When the water level drops about 8 cm during low tide, the intertidal wetland soil is exposed to the atmosphere.

The dilution tank which is placed on top of the mesocosm tank (Figure 1) is also made of acrylic with a size of about 22.5 cm x 10 cm x 15 cm to hold 1.2 L of clean salt water. The clean water from a storage tank (not shown in Figure 1) is pumped to the dilution tank through a pipe for about two minutes every day. The dilution tank is equipped with a float valve to assure a certain volume of water every time it fills. The water drips slowly (about 10 mL/min) from the dilution tank to the mesocosm tank to have enough mixing. When the drips stop (about 1 to 2 hours after they start), the water level in the mesocosm tank is still below an overflow outlet (Figure 1).

After a certain time (in this case about an hour after the dilution water starts dripping), the air pressure inside the air chamber increases due to the air pump activation. The increasing air pressure inside the air chamber pushes the water out to the mesocosm tank through the chute, resulting in an increase in the water level in the tank, simulating a rising tide. After the water inside the mesocosm tank reaches the overflow outlet (highest water level or tide), the excess water is flushed out from the tank to mimic the water flowing as washout or flushing. In this design, about 10% of the water volume inside the tank is discharged daily, representing a 10 day residence time typical of an open bay estuarine system (Wissel *et al*., 2005). The air volume inside the air chamber is controlled in order that the water level in the tank reaches the overflow outlet. The water flows out of the tank through the overflow outlet is due to the excess water from the dilution tank which has been mixed with the available water in the tank. Tidal movements in the mesocosm system can be simulated by controlling the air; for example, diurnally, that is half day at maximum and half day at minimum.

Several mesocosms can be controlled simultaneously using a master pneumatic tank which is built separately. The master tank also has an air chamber that works the same as the air chamber in the mesocosm tank. The air chambers of the replicate tanks and the master pneumatic tank are connected in parallel by some tubing to pass the air from the master to the replicates. Therefore, the air pressure can be distributed equally among the air chambers of the replicates. An increase in the air pressure in the pneumatic master causes air to flow displacing water upwards equally across the replicate mesocosms. A drop in pressure reverses the process.

EXPERIMENT USING THE LABORATORY MESOCOSM:

An experiment was run in the laboratory mesocosm to test the degradation of phenanthrene in a Louisiana coastal marsh wetland soil. The soil was taken from coastal marsh wetland near Empire, Louisiana (Lat. 29° 27.456'N, Long. 89° 46.841'W). Phenanthrene (a three-benzene-ring polycyclic aromatic), which is listed in priority pollutant list ATSDR, was spiked to the coastal marsh wetland soil in this experiment. Laboratory analysis result showed that the initial phenanthrene concentration in the contaminated wetland soil was 159.83 µg/kg (± 13.23 µg/kg). Two wetland soil trays which were filled with contaminated soil were placed above the top of the air chamber inside each of three mesocosm tanks (triplicate): one was higher than the other as shown in Figure 1. The higher tray simulated the intertidal wetland soil as the soil was intermittently exposed to the atmosphere due to tidal cycles; while the lower tray simulated the subtidal wetland soil as it was submerged all the time.

Sea salt water was made by mixing instant ocean sea salt mix with tap water to have artificial sea water with salinity of about 30 ppt. The tap water used in this study was originally disinfected with chloramine to a level of less than 1.4 mg-Cl_2/L (http://www.brwater.com/water-quality.html). Then the prepared sea water was aerated and aged for at least 24 hours prior to introduction to the dilution tank. No microorganism or nutrient was added to the water as the marsh wetland soil was assumed to contain both microorganism and nutrient for microbial activity. Tidal cycles applied in the system were to simulate water levels changing diurnally, half day high and half day low. The diurnal tidal cycles in this experiment followed: ten hours high tide, two hours down-slope tide, ten hours low tide, and two hours up-slope tide. Dilution water (about 10% of the water in the system) was added at a certain time every day by dripping it to the system at a flow rate of 10 mL/min. The excess water was discharged from an overflow hole to simulate flushing.

The experiment was run for about 15 weeks and a soil sample was taken weekly. Core sampling to collect about 2 mg of soil was performed using a clean straw and, then, another clean straw was placed in the sample hole. The PAH (in this case was phenanthrene) was extracted and the sample extract was analyzed on a HP 5890 GC/ 5972 series mass selective detector using EPA Method 8270.

RESULT AND DISCUSSION:

The coastal marsh wetland soil, both the subtidal and the intertidal, demonstrated a capacity to degrade phenanthrene. Three measurements of phenanthrene concentrations were made after 10 days, 1 month, 2 months, and 3 months on the subtidal and the intertidal wetland soil. The percent of phenanthrene removed was calculated. From these data the mean percent of phenanthrene removed and the standard deviations were calculated. Figure 3 shows the percent of phenanthrene removed from the subtidal wetland soil and the intertidal wetland soil of the laboratory mesocosm after 10 days, 1 month, 2 months, and 3 months. The initial concentrations of phenanthrene were the same in the two types of wetland soil (subtidal and intertidal). Even though both types of wetland soil have a capacity to remove phenanthrene, the results show that the phenanthrene removal rate in the subtidal wetland soil was slower. Phenanthrene was removed more any time in the intertidal wetland soil compared with that in the subtidal wetland soil (Figure 3). Greater degradation in the intertidal wetland soil is expected due to the oxygen resupply from the tidal cycles near the soil surface. Dissolved oxygen is assumed to be the primary electron acceptor in degrading organic compounds in the soil. The facultative condition of the intertidal wetland soil is favorable to microorganisms to degrade organic compounds. On the other hand, the subtidal wetland soil, which is always submerged, favors anaerobic microorganisms to utilize organic compounds.

Oxygen is the primary electron acceptor in aerobic systems for degrading phenanthrene even when the oxygen is available with other alternate electron acceptors. The renewal of oxygen in wetland soil is obtained after drainage of excess pore water during low tides. Due to the tidal cycles, the oxygen on the surface of the intertidal wetland soil is always renewed daily; thus, aerobic phenanthrene biodegradation can occur daily. The dissolved oxygen concentration can be measured in the water with a dissolved oxygen probe. The ORP (oxygen reduction potential) can be measured in the soil with an ORP probe and meter.

The laboratory mesocosm system can simulate the tidal cycles using the pneumatic system of the air chamber to manage the water levels in the experimental tanks. The types of the tidal cycle can be managed simply by arranging the activation and the deactivation time of the electrical air pump using a timer. The air chamber can also function as water storage chamber during low tides. The system can also be modified relatively easily, such as changing how low the lowest tide levels and how high the highest tide levels are. Also, by setting the timer which controls the activation and deactivation of the air pump, a certain type of tidal cycle can be simulated.

This designed mesocosm can be used as a tool to study the degradation of refractory organic compounds in the tidally influenced marsh wetland soils. This design is not intended to study direct oil contamination which may float or stick to the tank wall. However, it can be used to study several polycyclic aromatic hydrocarbons as major compounds of oil which present in the coastal marsh wetlands at the same time.

CONCLUSION:

The laboratory mesocosm developed in this study meets the objective as a tool to evaluate phenanthrene degradation in the subtidal and the intertidal wetland soil simulated in the system. The tidal water is recycled within the tank itself with the air chamber built inside the tank. The experimental results of phenanthrene degradations in the system follow typical first order reaction curves. As expected phenanthrene removal in the intertidal wetland soil was more and faster than that in the subtidal wetland soil. This laboratory mesocosm is suitable for non-volatile biodegradable compounds. It may be used for a small scale bioturbation studies

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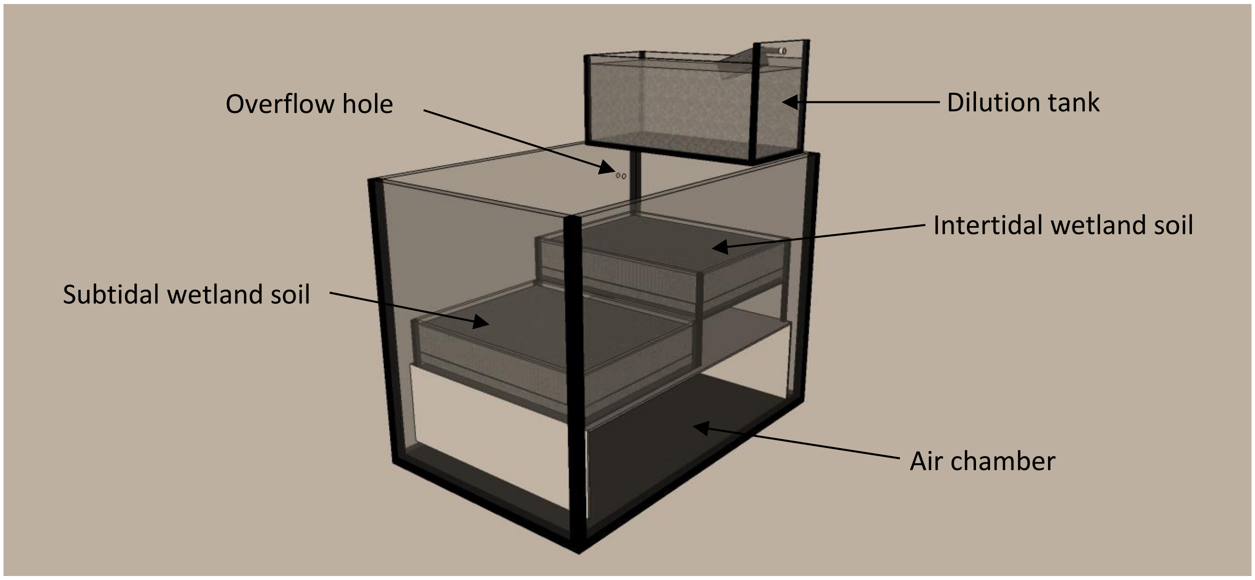


Figure 1. The schematic laboratory mesocosm of coastal marsh wetland

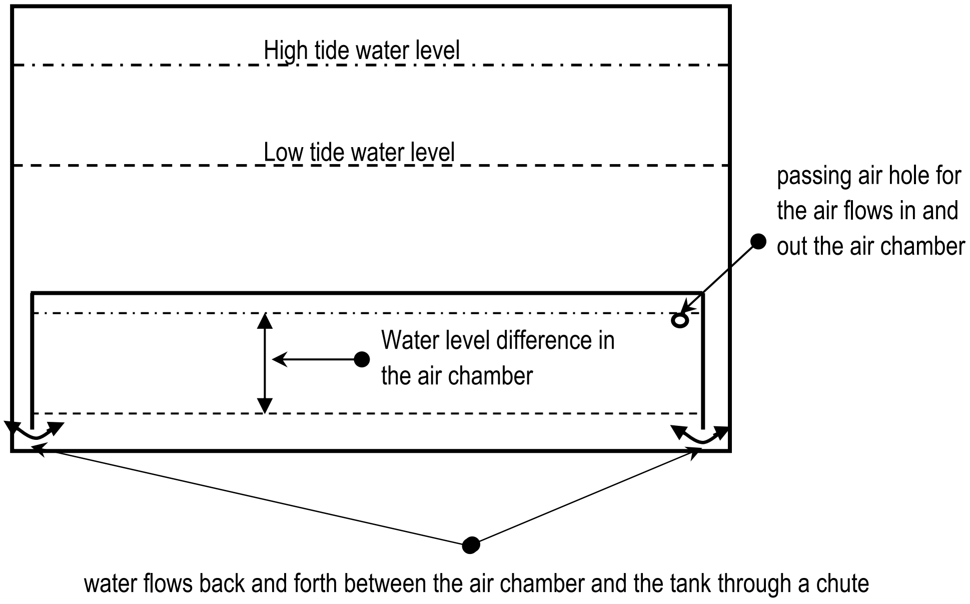
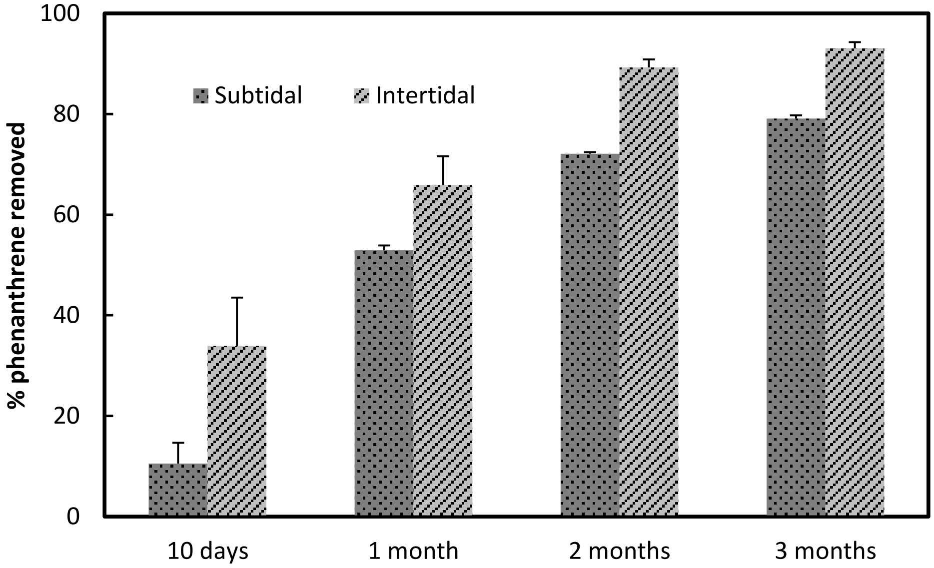


Figure 2. One side of mesocosm wall to show the air chamber inside the tank



Note: the error bars are standard deviations of triplicate measurements

Figure 3. The percent of phenanthrene removed from the subtidal and the intertidal wetland soil after 10 days, 1 month, 2 months, and 3 months