APPLICATION FORM
UNDERGRADUATE RESEARCH GRANT

Project Title: Evaluating polyaromatic hydrocarbon degrading microbial communities in *Spartina* marshes of the Great Bay Estuary, NH

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Abstract:

Degradation of polyaromatic hydrocarbons (PAHs) by microbial communities is an important area of microbiology which is far from being fully understood. PAHs are a class of highly toxic pollutants formed by the incomplete combustion of organic materials. Due to their chemical stability and poor water solubility, PAHs accumulate in the sediments of salt mash ecosystems. Salt marshes are an easily disrupted ecosystem, so removal of PAHs cannot be carried out by intrusive means (i.e. burning, tilling) and must rely on natural degradation by local microbial communities. While aerobic degradation of PAHs has been explored in studies past, the anaerobic processes involved in degradation are still much unknown. This study will look at both aerobic and anaerobic PAH degradation in conjunction with the influence of vegetation on the degradation process. This data will be gathered through the use of microcosm studies conducted in the laboratory after sediment samples have been collected from the salt marshes of the Cocheco River in Dover, NH.

Project Narrative:

Description of Proposed Project:

The goal of this project is to characterize PAH-degrading microbial communities in order to see the effects that vegetation with *Spartina alterniflora* and the presence of oxygen have on degrading abilities of these communities. In this study we will be using microcosms, which are a method whereby microorganisms in natural samples are grown in the laboratory using nutrient and incubation conditions expected to optimize growth and activity of the members of the PAH-degrading community. Samples will be taken from a PAH-contaminated salt mash containing areas vegetated with *Spartina alterniflora* and areas lacking any vegetation. Each type of sample will be cultured under aerobic and anaerobic conditions. The loss of PAH from the cultures will be monitored and compared to allow us to evaluate the effects of vegetation and oxygen status on the degradation process. In addition to evaluating the effect of the vegetation and oxygenation on microbial degradation, these microcosms will used in a future phase two of the project aimed at isolating and characterizing the genes used in the degradation process. This information will allow for the development of ways to track PAH-degrading microbial activity *in situ* with molecular methods. As depicted in figure 1, the idea is to use PAH degradation as an indicator of the number of PAH degrading bacteria. In this study we will focus on monitoring PAH degradation only. In future work we will also conduct microbial cell counts and measure the abundance of PAH-degradative genes.

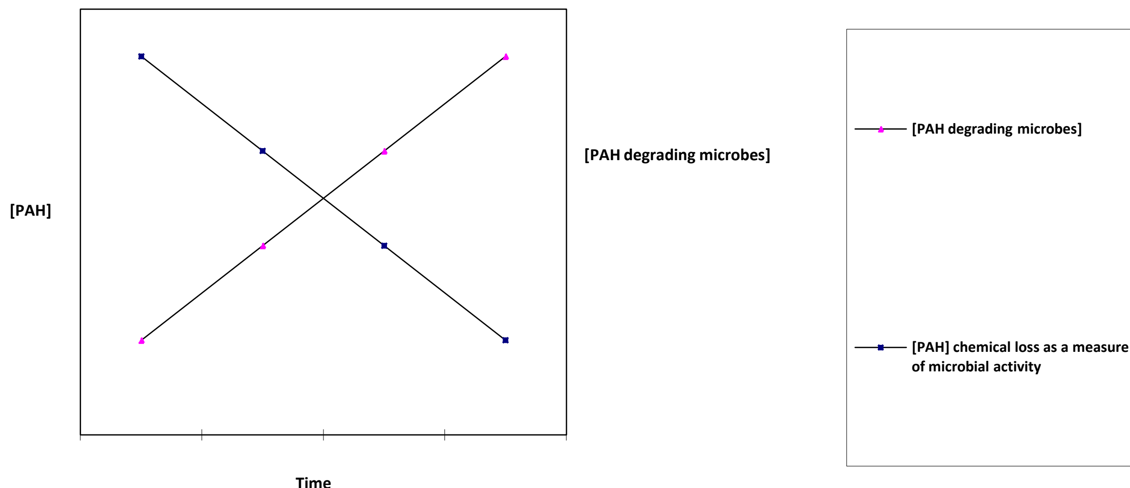

Figure 1. PAH degradation shown by the rise in microbial concentration and fall in PAH concentration over time.

Project Goals & Objectives:

1. Devise appropriate microcosm experimental design for comparing aerobic and anaerobic PAH degrading microbial communities and the effect of vegetation with *Spartina alterniflora*.
2. Conduct microcosm studies including establishment, maintenance sampling and PAH analysis.
3. Modify and apply analytical methods for PAH analysis to suit this project.
4. Organize and present project results at a regional undergraduate research meeting.

Background/Literature Review:

Polyaromatic hydrocarbons (PAHs) are a class of highly toxic pollutants often found in the sediment of salt marsh ecosystems. PAHs are formed from the incomplete combustion of organic materials. They deposit in the sediment due to their hydrophobic nature and stay in the sediment because of their chemical stability (Keck, J., et al. 1989, Van Metre, P.C. and B.J. Mahler 2005). Many PAHs are known mutagens (Bos, R.P., et al. 1988, Mersch-Sundermann, V., H.R. Rosenkranz, and G. Klopman 1992) and carcinogens (Buening, M.K., et al. 1978, Levin, W., et al. 1976) which have an impact on the local inhabitants, such as marine life and amphibians (Verhaar, H.J.M., C.J. van Leeuwen, and J.L.M. Hermens 1992). Salt marshes are an easily disrupted ecosystem which makes removal of PAHs by intrusive means, such as burning or tilling, not an option. One source of removal that has been observed is the natural degradation of PAHs by local microbial communities (Zhu, X., et al. 2004).

Microbes break down PAHs aerobically by use of enzymes called dioxygenases. Dioxygenases work by adding oxygen to the aromatic ring of a PAH followed by use of an electron transport chain to final catalysis of the PAH (Ni Chadhain, S.M., et al. 2006). Anaerobic degradation, however, is somewhat less understood. It takes place through a multistep process, seen in the PAHs naphthalene and phenanthrene, which is thought to begin with carboxylation or fumarate addition (Zhang, X.M. and L.Y. Young 1997, Musat F., et al. 2009, Sullivan, E.R., et al. 2001, Davidova, I.A., et al. 2007). Other evidence has shown that with naphthalene a different multistep process occurs, beginning with methylation of the naphthalene (Annweiler, E., et al. 2000, Annweiler, E., W. Michaelis, and R.U. Meckenstock 2002, Safinowski, M. and R.U. Meckenstock 2006).

There have been several studies conducted in the past pertaining to PAH-degradation by microbial communities. These studies have focused on the effects that temperature, nutrients, oxygen, pH, and salinity have on the degrading abilities of these communities. One promising area that has yet to be extensively studied is the effect of the presence or absence of vegetation on PAH-degradation (Launen, L.A., et al. 2008). As

previously discussed, there is also very limited knowledge on the process of anaerobic degradation which will be studied in conjunction with vegetation influences.

Methodology:

Experimental Design

Table 1: Aerobic Microcosm Design. Each cell indicates the number of vials to be sampled at a given time point and for a given sample type.

Sample Type	Time					
	t = 0	T – 3 days	t = 1 week	t = 2 weeks	t = 4 weeks	t = 8 weeks
Vegetated	*3	3	3	3	3	3
Not Vegetated	3	3	3	3	3	3
Vegetated/ Heat Killed	3	3	3	3	3	3
Not Vegetated/ Heat Killed	3	3	3	3	3	3

*For each time vs. sample type section, data from three different samples will be recorded.

Table 2: Anaerobic Microcosm Design. Each cell indicates the number of vials to be sampled at a given time point and for a given sample type.

Sample Type	Time					
	t = 0	t = 3 weeks	t = 6 weeks	t = 9 weeks	t = 12 weeks	t = 15 weeks
Vegetated	*3	3	3	3	3	3
Not Vegetated	3	3	3	3	3	3
Vegetated/ Heat Killed	3	3	3	3	3	3
Not Vegetated/ Heat Killed	3	3	3	3	3	3

*For each time vs. sample type section, data from three different samples will be recorded.

Sampling Design

We will be taking samples for this project from the *Spartina alterniflora* inhabited salt Cocheco River site in Dover, NH. Sampling will be done during low tide from areas that are regularly flooded during high tide. Samples will be collected from areas with and without vegetation. From each area samples will be collected from several areas that are vegetated or unvegetated. Each sample will consist of a core taken from surface to 15 cm below surface. The core will be approximately 30 cm wide. Each sample will be subdivided as follows: 1) microcosm setup 2) frozen for later DNA analysis 3) used for PAH analysis. The samples will be kept on ice during transport to the laboratory where the samples for salinity and pH will be assessed immediately while the samples for microbial parameters and PAH concentrations will be stored in a freezer at -80°C.

Microcosm Setup

Microcosms will be setup using 60 mL serum vials containing 5 g of sediment in a total volume of 15 mL Modified Marine Salts Medium (MSM) for aerobic incubation and 15 g of sediment in a total volume of 45 mL MSM for anaerobic incubation. Anaerobic microcosms will be filled with a 30:70 N_2:CO_2 mixture to rid the vials of oxygen at the start. The vials will be sealed with a rubber stopper and any remaining oxygen will be

removed by aerobic microbial activity. To assure anaerobic conditions are maintained, resazurin will be used as a redox indicator to monitor conditions. Aerobic microcosms will be amended with naphthalene and phenanthrene [200 mg/L]. Anaerobic microcosms will be amended with naphthalene and 2-methylnaphthalene [200 mg/L]. For each microcosm, three vials will be kept (for both aerobic and anaerobic) and checked at specific time points. PAH degradation will be monitored over time. Each microcosm vial when sampled will be split as follows: 1/3 extracted for PAH analysis 1/3 frozen for later DNA analysis 1/3 frozen for later cell counts.

PAH Analysis

The sediment samples for PAH contamination will be extracted with a 1:1 hexane: acetone mixture. PAH levels of the extracts will be analyzed by GC-MS using a method based on EPA Method 8270C and 2,3-dimethyl naphthalene as an internal standard once it is verified that this compound is not present in the sampled sediments. The GC-MS system is a Shimadzu GCMS-QP5050A (GC is GC-17A) equipped with a Restek RTX-XLB 30 m, 0.25 mm diameter column.

Project Timeline:

Objective	Time	Accomplished
Write undergraduate research grant, develop plan	Sep-09	Completed
Sample and establish microcosms	Oct-09	Completed
Adjust and verify PAH analytics	Oct/Nov-09	Completed
Sampling microcosm setup	Oct - Jan-09-10	In Progress
Analysis of microcosm samples	Dec - Jan-09-10	No
Data analysis	Jan - Feb-10	No
Poster/ talk preparation	Feb/Mar-10	No
Poster/ talk Presentation (NURDS, AEC)*	Mar-10	No

*NURDS – Northeast Undergraduate Research Development Symposium
AEC – Academic Excellence Conference

Project Budget:

Item	Supplier	Amount	Cost	Total
GC-MS vials w/ caps	Fisher Scientific/Thermo Orion	1	\$150.00	\$150.00
Acetone (4 L bottle)	Fisher Scientific/Thermo Orion	1	\$156.00	\$156.00
Hexane (4 L bottle)	Fisher Scientific/Thermo Orion	1	\$193.53	\$193.53
		Shipping		\$25.00
		Total		\$524.53

Project Budget Justification:

All supplies are for analysis of samples using GC-MS, which will be conducted in December and January of 09/10. GC-MS vials and caps are used to put samples in for testing by use of the gas chromatography mass spectrometer. The N_2/CO_2 tank is needed to remove oxygen from the anaerobic portions of the experiment. Acetone and Hexane are solvents that will be used throughout the project.

Use of Human Subjects or Vertebrate Animals:

Does your project include the use of human subjects for research? NO
Does your project include the use of other vertebrate animals? NO

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