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Bacterial Metabolism, Aromatic Biodegradation, and Lignin Biogeochemistry in Sediment Cores from Pearl Harbor, Hawaii

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Heterotrophic bacteria require a source of oxygen to rapidly metabolize complex and recalcitrant carbon sources like lignin, 2,4,6 trinitrotoluene (TNT), and polycyclic aromatic hydrocarbons (PAHs). The activities of burrowing macrofauna can increase oxygenation, which may stimulate bacterial metabolism of PAHs and heterotrophic production. We measured bacterial production, PAH mineralization, and lignin subunit concentration with depth in cores taken from South Loch and Bishop's Point in Pearl Harbor, Hawaii. Bacterial metabolism decreased rapidly with depth but was much higher at South Loch than Bishop's Point. PAH mineralization rates were higher at South Loch than at Bishop's Point and extended down to the depths of bioturbation at each site: upper 4 to 6 cm at Bishop's Point and upper 9 cm at South Loch. Phenolic moieties of lignin at South Loch and Bishop's Point suggest there are different sources of organic matter to the two sites. PAH mineralization was elevated in bioturbated zones from both sites relative to those below this zone. Ambient PAH concentrations were also higher at the less bioturbated site. This is consistent with the hypothesis that benthic infauna stimulate PAH metabolism.							
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EXECUTIVE SUMMARY

Heterotrophic bacteria require a source of oxygen to rapidly metabolize complex and recalcitrant carbon sources like lignin, 2,4,6 trinitrotoluene (TNT), and polycyclic aromatic hydrocarbons (PAHs). Natural bacterial assemblages can rapidly deplete oxygen in marine sediments that depend primarily on diffusive processes for aeration. However, the activities of burrowing macrofauna can increase oxygenation, which may stimulate bacterial metabolism of PAHs and heterotrophic production. In this study, we measured bacterial production, PAH mineralization, and lignin subunit concentration with depth in cores taken from more bioturbated (South Loch) and less bioturbated (Bishop's Point) stations in Pearl Harbor, Hawaii. Rates of bacterial metabolism decreased rapidly with depth but were much higher at South Loch than at Bishop's Point. Similarly, PAH mineralization rates were higher at South Loch than at Bishop's Point and extended down to the depths of bioturbation at each site: upper 4 to 6 cm at Bishop's Point and upper 9 cm at South Loch. Comparing the total concentration and ratio of phenolic moieties of lignin in the sediments of South Loch and Bishop's Point suggests there are different sources of organic matter to the two sites. Pearl Harbor is heavily urbanized and much of the angiosperm signal at the South Loch may be due to upland drainage in the subwatershed, whereas Bishop's Point is near the mouth of the harbor and likely has marine dilution of the lignin signal in the sediments. In general, the lignin appeared more degraded in the top 9 cm at all stations sampled and was more degraded at South Loch relative to Bishop's Point. We found that PAH mineralization was elevated in the bioturbated zones from both stations relative to core subsections from below the bioturbated zone. In addition, ambient PAH concentrations were higher at the less bioturbated site. This is consistent with the hypothesis that the activities of benthic infauna stimulate bacterial metabolism of PAHs.

BACTERIAL METABOLISM, AROMATIC BIODEGRADATION, AND LIGNIN BIOGEOCHEMISTRY IN SEDIMENT CORES FROM PEARL HARBOR, HAWAII

INTRODUCTION

Heterotrophic bacteria require a source of oxygen to rapidly metabolize complex and recalcitrant carbon sources like lignin, 2,4,6-trinitrotoluene (TNT), and polycyclic aromatic hydrocarbons (PAHs). Bacterial assemblages can rapidly deplete oxygen in marine sediments that depend primarily on diffusive processes for aeration. However, as we reported in a previous study on San Diego Harbor sediments (Montgomery et al. 2003), the activities of burrowing macrofauna can stimulate bacterial metabolism of PAHs and heterotrophic production. In this follow-up study, we also measured heterotrophic bacterial production, PAH mineralization, and lignin subunit concentration with depth in cores taken from bioturbated and less bioturbated stations in Pearl Harbor, Hawaii.

MATERIALS AND METHODS

Sampling

Replicate gravity cores housed on a multicorer were sampled from two stations at South Loch and two stations at Bishop's Point in Pearl Harbor, Hawaii. Stations at South Loch (SLA, SLC) were sampled on 17 December 2002, and stations at Bishop's Point (BPB, BPC) were sampled on 18 December 2002. The multicorer was deployed off a small research vessel and was transferred to the laboratory at ambient temperature within 3 hours. Two cores from South Loch stations (SLA, SLC) and Bishop's Point stations (BPB, BPC) were sectioned and assayed for bacterial production and PAH mineralization while a third replicate core from each station was sectioned for PAH and lignin subunit concentration. Slurries for biological assays were made from filtered water overlying the respective cores.

Heterotrophic Bacterial Production

The leucine incorporation method (Kirchman et al. 1985, Kirchman 1993, Smith and Azam 1992) was used to measure bacterial production as adapted by Montgomery et al. (1999). A 0.50 μ L aliquot of wet surface sediment from each station was added to 2 mL microcentrifuge tubes (three experimental and one control), which were precharged with [³H-4,5]-L-leucine (154 mCi mmol⁻¹). The sediment was extracted from the benthic grab sample and was added to the 2 mL tube using a 1 mL plastic syringe with the end cut off. One mL of 0.45 μ m nom. pore dia. (Acrodisk, Gelman) filtered bottom water (collected <1 m above bottom) was then added to each tube to form a sediment slurry. Samples were incubated for 1 to 2 hours at in situ temperatures and were subsequently processed by the method of Smith and Azam (1992). A constant isotope dilution factor of 2 was used for all samples. This was estimated from actual measurements of sediment dissolved free amino acids (Burdige and Martens 1990) and saturation experiment estimates (Tuominen 1995). One mL syringed samples of wet sediment were dried at 50 °C and were used to convert production values from wet weight to dry weight. Leucine incorporation rate was converted to bacterial carbon using factors determined by Simon and Azam (1989).

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PAH Concentration

Ambient PAH concentrations of 18 semivolatile priority pollutants were determined. First, 10 to 15 g of sediment were dried with diatomaceous earth and were then extracted in methanol using accelerated solvent extraction. The extracts were concentrated under a N_2 stream (Speedvap) and were analyzed by GC/MS (Fisher et al. 1997). Following the method described in Pohlman et al. (2002), *p*-Terphenyl-d₁₄ and 2-fluorobiphenyl were used as surrogate standards.

Radiotracer Mineralization

Radiotracer mineralization assays were initiated within three hours of sediment sample collection using a modification of Boyd et al. (1996) and Pohlman et al. (2002). For radiotracers, we used three sentinel PAHs: UL-¹⁴C-naphthalene (18.6 mCi mmol⁻¹), 3-¹⁴C-fluoranthene (45 mCi mmol⁻¹), and 9-¹⁴C-phenanthrene (47 mCi mmol⁻¹), which were purchased from Sigma Chemical. 2,4,6-¹⁴C-TNT (50 mCi mmol⁻¹) and U-¹⁴C-catechol (3.5 mCi mmol⁻¹) were purchased from American Radiochemical Chemicals Inc. They were added in separate incubations to surface sediment samples (1 mL wet volume) in 100 × 16 mm test tubes to a final concentration of about 0.2 μ g g⁻¹ (depending on specific activity). Isotope dilution of PAHs was calculated from the ambient PAH concentration, and additions were intended to be < 10% of ambient PAH concentration to minimize selective pressure on the natural bacterial assemblage. Ambient catechol and TNT concentrations were expected to be below detection and were not measured. Thus, these compounds are not treated as tracers of ambient pool mineralization but rather potential for assemblage utilization. Samples were incubated no longer than 24 h at in situ temperature, and evolved ¹⁴CO₂ was captured on NaOH-soaked filter papers suspended in the headspace of each tube. H₂SO₄ was added to end incubations and to partition any remaining CO₂ into the headspace of the tube and to the filter paper trap. The filter paper traps containing metabolized ¹⁴CO₂ were removed, radioassayed, and subsequently used to calculate substrate mineralization.

Lignin Concentration

Lignin concentration in sediment samples was measured using an alkaline hydrolysis oxidation method to liberate lignin-derived methoxyphenols derived from the parent lignin compound (Table 1) as previously described by Montgomery and Osburn (2003). These compounds were subsequently derivatized with 1% BSTFA to silyate exchangeable hydrogen and were then analyzed by GC/MS (Goni and Montgomery 2000). Compounds were separated using a J&W Scientific DB-1 column (60 m × 0.32 mm i.d., 0.2 μ m film thickness) with the following analytical program: 100 °C initial temperature, 4 °C/min temperature ramp, 320 °C final temperature, and final hold of 10 min. A splitless, on-column injector with a flow rate of 1.3 mL min⁻¹ mode was used for the GC. MS spectra of eluted peaks were interpreted using an internal laboratory library that was created based on the retention times and m/z values for standards purchased from Sigma-Aldrich. Acid, aldehyde, and ketone moieties of phenols liberated from the lignin hydrolysis and oxidation provide useful geochemical information about terrigenous organic carbon derived from vascular plants in coastal sediments (Hedges and Ertel 1982). These moieties can be used to describe the collective geochemical history of the terrigenous organic carbon (tissue source, diagenetic history) and provide a context (in addition to contaminant concentration and speciation) in which to interpret microbial activity in sediments (Table 1).

RESULTS

Heterotrophic bacterial production was measured at two stations at Bishop's Point, BPB and BPC, and at two stations at South Loch, SLA and SLC. Production remained above 3.3 μ g C kg⁻¹ d⁻¹ in core sections representing the top 9 cm of both South Loch stations (Fig. 1) and was as high as 30.9 μ g C kg⁻¹ d⁻¹ in the top 2 cm at station SLA. Bacterial metabolism was much lower at the Bishop's Point

Parameter	Definition	Significance	
V	Vanillyl family	Synthesized in all vascular plants	
S	Syringyl family	Synthesized only in angiosperms	
С	Cinnamyl family	Synthesized only in nonwoody tissues (leaves, needles)	
S/V	Ratio of syringyl to vanillyl phenols	Values > 0 if large contribution of angiosperms	
C/V	Ratio of cinnamyl to vanillyl phenols	Values = 0 if gymnosperm wood; >0 if gymnosperm needles	
Λ	mg phenol per 100 mg of organic carbon (OC)	Lignin normalized to OC content of sediments	
[ad/al] _v	Ratio of acid to aldehyde moieties in vanillyl family	Values > 0.5 indicate oxidative degradation (microbial); (Opsahl and Benner 1995)	

Table 1 — Summary of Lignin-derived Phenol Measurements and Parameters

stations being only slightly above 5 μ g C kg⁻¹ d⁻¹ (BPB, 5.8; BPC, 5.9) in the top 2 cm with the 2 to 4 cm slightly lower (BPB, 2.7; Fig. 2). Values for the stations at each site were similar to each other in pattern of decrease with depth and in absolute value for each core section. Overall, bacterial production at South Loch was higher than that for corresponding depths at Bishop's Point.

Total PAH concentration was below 2 ppm for all core sections at both South Loch stations (Fig. 3), and changes were generally unremarkable with depth though the deepest section was also the lowest in PAH concentration (SLA, 0.88; SLC, 0.74). At Bishop's Point, PAH concentration was much higher than at South Loch in all core sections at station BPC and was highest in the top 0 to 2 cm at BPB (10.13 ppm; Fig. 4). At BPC, PAH concentration generally decreased with depth, whereas BPB had the second highest concentration (6.23 ppm) in the deepest core section (9 to 13 cm).

Radiotracer mineralization rates of three sentinel PAHs were measured to determine how rapidly the ambient pool of PAH was being metabolized by the heterotrophic bacterial assemblage. At Bishop's Point station BPB, naphthalene and fluoranthene mineralization rates were relatively low throughout all core sections but highest in the top 0 to 2 cm (Fig. 5), as expected for this oxygen utilizing process. Phenanthrene mineralization rates were generally highest of the three PAHs measured and were elevated in the upper 0 to 4 cm of the BPB cores. At Bishop's Point station BPC, PAH mineralization was low in the top 0 to 2 cm and the bottom 9 to 13 cm (Fig. 6), which were also the depths of highest ambient PAH concentration for all cores (Fig. 4 vs Fig. 3). Phenanthrene and fluoranthene mineralization was highest from core sections representing 2 to 9 cm below the bottom water-sediment interface. PAH mineralization rates at both South Loch stations were generally higher than at corresponding depths for Bishop's Point cores and generally highest in the top 0 to 6 cm (Figs. 7, 8). Phenanthrene mineralization was often higher in each core section than that for naphthalene and fluoranthene (Figs. 7, 8). Catechol mineralization was generally highest in core sections representing 2 to 9 cm deep and was similar among all four stations (Fig. 9). This pattern contrasts with that for total heterotrophic production (Figs. 1, 2), suggesting that catechol mineralization rates may be associated with aromatic metabolism rather than a simple function of carbon metabolism, in general. TNT mineralization rates were generally higher in South Loch core sections from both stations than those for corresponding sections from Bishop's Point stations, but did not show distinct differences with depth at any station (Fig. 10).

Lignin phenol distributions in the sediment cores were used to assess the refractory natural organic material buried in sediments. The syringyl to vanillyl (S/V) vs cinnamyl to vanillyl (C/V) ratios suggested that the source of terrigenous organic matter and the type of vascular tissue were different at each site. South Loch has more influence from angiosperm tissue than does Bishop's Point, especially relative to station

BPB (Fig. 11). At Bishop's Point, there was low abundance of syringyl phenols and no cinnamyl phenols; further, organic carbon-normalized lignin concentrations (Λ) were about one third those from Bishop's Point (Fig. 12). In terms of diagenetic state, or reactivity, both sites are remarkably similar in the top 0 to 6 cm with [ad/al]_v ratios 0.7 or above indicating that the lignin was relatively degraded (Fig. 13). Below 6 cm, core sections from station BPC appeared less degraded than those from the three other stations. Finally, concentration of cinnamyl phenols relative to organic carbon was higher at South Loch than at Bishop's Point. Station SLC had even higher total cinnamyl concentration than SLA (Fig. 14), and the total cinnamyl concentration, as well as the C/V ratio, both showed some correlation with fluoranthene turnover at South Loch (Fig. 15).

DISCUSSION

This study compared the depth related changes in bacterial metabolism of organic matter in the sediments of two sites in Pearl Harbor—Bishop's Point and South Loch. The two sites were chosen to be different from each other based on their degree, types, and depth of bioturbation, with South Loch being the more bioturbated of the two sites (as determined using REMOTS camera analyses). Two coring stations at each site were selected to address the variability at each station. Our previous two studies at Bishop's Point found large changes in PAH concentration and rapid PAH turnover in the surface sediments (Montgomery et al. 2002). These changes in ambient PAH concentration may be due to creosote treatment of docks or the presence of a refueling dock. The PAH turnover and mineralization rate by bacteria was among the most rapid that our group has reported for any estuarine site and may be due to bacterial assemblage adaptation to episodic inputs of petroleum products (Montgomery et al. 2002). In this study, we found that PAH concentrations in the surface sediments were much lower than during our previous samplings in 1998 and 1999, but higher than found in our related study of Paleta Creek in San Diego Bay (Montgomery et al. 2003).

Rates of total bacterial metabolism decreased rapidly with depth but were much higher at South Loch than at Bishop's Point. Similarly, PAH mineralization rates were higher at South Loch than at Bishop's Point and extended down to the likely depths of bioturbation at each site: upper 4 to 6 cm at Bishop's Point and upper 9 cm at South Loch. These findings are consistent with the hypothesis furthered in our previous study in San Diego Bay, that the burrowing and irrigational activities of macrofauna can stimulate bacterial degradation of PAHs in sediments that would otherwise be anoxic (Montgomery et al. 2003). One odd finding was that both production and PAH mineralization were very low in the top 0 to 2 cm of Bishop's Point station, BPC, but then mineralization was much higher from 2 to 6 cm. One hypothesis is that the surface sediments (0 to 2 cm) were relatively armored but that tube forming macrofauna were oxygenating deeper into the sediments but not affecting the surface itself.

Catechol is a relatively labile compound but it may represent an intermediate in the metabolism of many aromatics such as PAHs, lignin, and more exotic aromatics, like TNT. That is, bacterial assemblages, that can rapidly degrade more recalcitrant aromatics, may mineralize catechol more rapidly than assemblages that are predominantly metabolizing nonaromatic carbon sources. For both sites, rates of catechol activity did not directly correspond to rates of production (which were highest in the top section), as they would if catechol mineralization rate was a general function of total metabolic activity. Rather, the rates were higher in the upper 9 cm of both sites corresponding to depths of enhanced PAH metabolism and the presence of more degraded lignin. Thus, rapid catechol mineralization may be more indicative of a bacterial assemblage that is metabolizing aromatic carbon sources to support production. In addition, TNT metabolism was much higher in the more bioturbated site but did not show a distinct pattern with depth. In terrestrial systems, both aerobic and anaerobic metabolic processes are used for complete mineralization of TNT, and this may also be a requirement for marine systems. The need of the bacterial assemblage for alternating aerobic and anaerobic microenvironments may have led to our observed depth profile differences between South Loch and Bishop's Point.









0.0E+00 5.0E-03 1.0E-02 1.5E-02 2.0E-02 2.5E-02 3.0E-02 3.5E-02

Fig. 8 — Mineralization ($\mu g \ C \ kg^{-1} \ d^{-1}$) of the PAHs, naphthalene (**I**), phenanthrene (**O**), and fluoranthene (Δ) vs depth (cm) in core sections taken at station SLC3 in South Loch.







Fig. 10 — TNT mineralization ($\mu g C k g^{-1} d^{-1}$) vs depth (cm) in core sections taken at stations BPB2 (\blacksquare), BPC6 (\bullet), SLA2 (Δ), and SLC3 (\Box), in Bishop's Point and South Loch.



Fig. 11 — Lignin-derived phenol ratios (syringyl: vanillyl, S/V; cinnamyl: vanillyl, C/V) in core sections taken at stations BPB2 (\Box), BPC6 (Δ), SLA2 (\bullet), and SLC3 (\blacksquare) in Bishop's Point and South Loch.



Fig. 12 — Organic carbon normalized lignin distribution (Σ_8 , mg/100 mg OC) with depth (cm) in core sections taken at BPB2 (Δ), BPC6 (\Box), SLA2 (\blacksquare), and SLC3 (\bullet) in Bishop's Point and South Loch.



Fig. 13 — Ratio of acid to aldehyde phenolic moieties in the vanillyl family ([ad/al]v) vs depth (cm) in core sections taken at BPB2 (Δ), BPC6 (\Box), SLA2 (\blacksquare), and SLC3 (\bullet) in Bishop's Point and South Loch.



Fig. 14. — Concentration of cinnamyl phenols (\blacksquare ; mg/100 mg OC) and ratio of cinnamyl to vanillyl phenols (\bigcirc ; C/V) vs depth (cm) in core sections taken at the two stations in Pearl Harbor, HI.



Fig. 15 — Relationship between fluoranthene turnover (days), cinnamyl phenol concentration (\circ ; mg/100 mg OC), and C/V ratio (\blacksquare) in South Loch.

A second component of this study involved assessing the biogeochemistry of lignin and relating this to bacterial metabolism of aromatic organic matter in the sediments. Comparing the total concentration and ratio of phenolic moieties of lignin in the sediments of South Loch and Bishop's Point suggests that there are different sources of organic matter to the two sites. Pearl Harbor is heavily urbanized and probably much of the angiosperm signal at the South Loch may be due to upland drainage in the sub-watershed, whereas Bishop's Point is near the mouth of the harbor and likely has marine dilution of the lignin signal in the sediments. In general, based on the $[ad/al]_v$ and the C/V ratios, the lignin appeared more degraded in the top 9 cm at all stations and at South Loch relative to Bishop's Point. These results are consistent with preferential removal of vanillyl phenols during the degradation of lignin (Goni et al. 1993, Hedges et al. 1988). Some correlation was found between the C/V ratio and fluoranthene turnover time, which suggests that there may be some relationship between PAH degradation and source tissue of lignin. One should caution that the fluoranthene turnover measurements and the biogeochemical processes that generated the lignin phenol ratios occur over dramatically different time scales. Catechol mineralization was highest from 2 to 9 cm below the bottom water suggesting that aromatic compounds may be playing a larger role in supporting bacterial production in the bioturbation zone below the upper few cm than at other depths.

We found that PAH mineralization was elevated in the bioturbated zones from both sites relative to core subsections from below the bioturbated zone. In addition, ambient PAH concentrations were higher at the less bioturbated site. This is consistent with the hypothesis that the activities of benthic infauna stimulate bacterial metabolism of PAHs.

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REFERENCES

- Boyd, T. J., B. J. Spargo, and M. T. Montgomery. 1996. "Improved Method for Measuring Biodegradation Rates of Hydrocarbons in Natural Water Samples," in *In Situ Bioremediation and Efficacy Monitoring*, B. J. Spargo, ed., NRL/PU/6115-96-317, pp. 113-121.
- Burdige, D. J. and C. S. Martens. 1990. "Biogeochemical Cycling in an Organic-Rich Marine Basin 11. The Sedimentary Cycling of Dissolved Free Amino Acids," *Geochim. Cosmochim. Acta* **54**: 3033-3052.
- Fisher, J. A., M. J. Scarlett, and A. D. Stott. 1997. "Accelerated Solvent Extraction: An Evaluation for Screening of Soils for Selected U.S. EPA Semivolatile Organic Priority Pollutants," *Environ. Sci. Technol.* 31: 1120-1127.
- Goni, M. A. and S. Montgomery. 2000. "Alkaline CuO Oxidation with a Microwave Digestion System: Lignin Analyses of Geochemical Samples," *Anal. Chem.* **72**: 3116-3121.
- Goni, M. A., B. Nelson, R. A. Blanchette, and J. I. Hedges. 1993. "Fungal Degradation of Wood Lignins -Geochemical Perspectives from CuO-derived Phenolic Dimers and Monomers," *Geochim. Cosmochim. Acta* 57(16): 3985-4002.
- Hedges, J. I., R. A. Blanchette, K. Weliky, and A. H. Devol. 1988. "Effects of Fungal Degradation on the CuO Oxidation Products of Lignin - a Controlled Laboratory Study," *Geochim. Cosmochim. Acta* 52(11): 2717-2726.
- Hedges, J. I. and J. R. Ertel. 1982. "Characterization of Lignin by Gas Capillary Chromatography of Cupric Oxide Oxidation-Products," Anal. Chem. 54(2): 174-178.
- Kirchman, D. L. 1993. "Leucine Incorporation as a Measure of Biomass Production by Heterotrophic Bacteria," pp. 509-512, In: *Handbook of Methods in Aquatic Microbial Ecology*, P. F. Kemp, B. F. Sherr, E. B. Sherr, and J. J. Cole, eds. (Lewis Publishers, Ann Arbor), 777 pp.
- Kirchman, D. L., E. K'Nees, and R. Hodson. 1985. "Leucine Incorporation and Its Potential as a Measure of Protein Synthesis by Bacteria in Natural Aquatic Systems," *Appl. Environ. Microbiol.* **49**: 599-607.
- Montgomery, M. T., T. J. Boyd, B. J. Spargo, R. B. Coffin, J. K. Steele, D. M. Ward, and D. C. Smith. 1999.
 "Bacterial Assemblage Adaptation in PAH-impacted Ecosystems." in: *In Situ and On-Site Bioremediation*, B. C. Alleman and A. Leeson, eds. (Battelle Press, Columbus, OH), 5(8): 223-228.
- Montgomery, M. T. and C. L. Osburn. 2003. "Depth Profile of Bacterial Metabolism and PAH Biodegradation in Bioturbated and Unbioturbated Marine Sediments," NRL/FR/6114--03-10,057.
- Montgomery, M. T., C. L. Osburn, W. Zeibis, C. Mahn, and J. M. Gieskes. 2003. "Bacterial PAH Metabolism with Depth in Marine Sediments," *Marine Environ. Research (in submission April 2004)*.
- Montgomery, M. T., D. C. Smith, C. L. Osburn, and T. J. Boyd. 2002. "Bacterial Degradation of Aromatic Hydrocarbons in Surface Sediments of Temperate and Tropical Coastal Ecosystems," *Eos. Trans. Amer. Geophys. Union* 83(4): OS21O-06.

- Opsahl, S. and R. Benner. 1995. "Early Diagenesis of Vascular Plant-Tissues Lignin and Cutin Decomposition and Biogeochemical Implications," *Geochim. Cosmochim. Acta* **59**(23): 4889-4904.
- Pohlman, J. W., R. B. Coffin, C. S., Mitchell, M. T. Montgomery, B. J. Spargo, J. K. Steele, and T. J. Boyd. 2002. "Transport, Deposition, and Biodegradation of Particle Bound Polycyclic Aromatic Hydrocarbons in a Tidal Basin of an Industrial Watershed," *Environ. Monitor. Assess.* 75: 155-167.
- Simon, M. and F. Azam. 1989. "Protein Content and Protein Synthesis Rates of Planktonic Marine Bacteria," *Mar. Ecol. Prog.* Ser. **51**: 201-213.
- Smith, D. C. and F. Azam. 1992. "A Simple, Economical Method for Measuring Bacterial Protein Synthesis Rates in Seawater using ³H-leucine," *Mar. Microb. Food Webs* **6**(2): 107 114.
- Tuominen, L. 1995. "Comparison of Leucine Uptake Methods and a Thymidine Incorporation Method for Measuring Bacterial Activity in Sediment," *J. Microbiol. Methods* 24: 125-134.