Coastal Marine Institute

Biodegradation of Aromatic Heterocycles from Petroleum-Produced Water and Pyrogenic Sources in Marine Sediments

Final Report

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

Cooperative Agreement
Coastal Marine Institute
Louisiana State University

Coastal Marine Institute

Biodegradation of Aromatic Heterocycles from Petroleum-Produced Water and Pyrogenic Sources in Marine Sediments

Final Report

Authors

W. James Catallo

August 2000

DISCLAIMER

REPORT AVAILABILITY

Extra copies of this report may be obtained from the Public Information Office (Mail Stop 5034) at the following address:

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region
Public Information Office (MS 5034)
1201 Elmwood Park Boulevard
New Orleans, LA 70123-2394

Telephone: 504-736-2519 or 1-800-200-GULF

CITATION

Catallo, W. J. 2000. Biodegradation of Aromatic Heterocycles from Petroleum-Produced Water and Pyrogenic Sources in Marine Sediments. OCS Study MMS 2000-060. U.S. Department of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, LA. 28 pp.

ACKNOWLEDGEMENTS

EXECUTIVE SUMMARY

This report summarizes laboratory studies and associated technical development on the influence of hydrology on biogeochemical processes, particularly those that govern the transport and transformation of pollutant organic chemicals in wetland sediments. The goals were to: 1) determine the effects of static vs. dynamic hydrology on biogeochemical behavior of tidal wetland sediment systems, with respect to central variables (e.g., redox potential dynamics) and pollutant transformation, 2) design and build hydrodynamic microcosms for this purpose that are reproducible, well controlled, and adequately monitored in the laboratory and, 3) develop analytical approaches for target pollutant chemicals that allow for detection of small but significant concentration differences between time points and treatments. This last goal was included because most common methods used for identification of chemicals mixtures in sediments are semi-quantitative (i.e., provide for order-of-magnitude differentiation in analyte concentrations), and it was expected that differences in target pollutant levels between treatments in this work would be inside this margin and not discernable using standard approaches. The pollutant chemicals examined here included representative N-, O-, and S- heterocycles (NOSHs) that occur in coal chemicals, crude oils, and pyrogenic mixtures. The target analytes were quinoxaline, 2-methylquinoxaline, 2,3-dimethylquinoxaline, benzothiazole, dibenzofuran, phenazine, dibenzothiophene, phenothiazine, acridine, and phenanthridine. These and other NOSHs are found in in pollutant mixtures near certain industrial activity, hazardous waste sites, and major harbors. They are of interest because of their apparent stability in aquatic sediments except for certain (turbulent, well-oxidized) conditions under which they are labile. Further, many NOSH compounds are acutely toxic, with some also mutagenic and/or carcinogenic in mammals. They were synthesized in deuterated form using approaches developed for this and related work, and used as isotopic dilution standards in GC-MS analysis of sediments collected from experimental sediments over some 20 weeks. The sediments were exposed to three hydrologic conditions: static drained (oxidized redox potentials), static flooded (reduced redox potentials), and tidal (alternating redox potential). It was found that the physcochemical and biological behavior of the sediments in the microcoms was similar to that observed in natural settings, and in accord with prevailing theory. Redox potential time series and sediment trace gas signatures from the different treatments were analogous to those encountered in field settings continuously flooded and drained systems had reduced (mean - 428 mV) and oxidized (+ 73 mV) Eh values, respectively, with no evidence of daily or longer periodic variation. The tidal systems exhibited oscillating Eh values, with significant amplitudes (40 – 180 mV/0.5 d). Analysis of the signals using the wavelet transform confirmed the presence of strong diurnal Eh variations, with a mean value period of 23.78 ± 2.10 h. Thermal and light cycles in the laboratory had little effect of the Eh time signature: variation of tide stage throughout the day and at different times of the year (the experiments were conducted in an annex not equipped with climate control) did not affect the tidal Eh signal period. Biogeochemical gas mixtures leaving the sediments also reflected the hydrological status of the treatments: the major gases observed were CO_2 from microbial respiration (drained) vs. CO_2 with small sulfide and methane outputs (tidal), vs. relatively large amounts of sulfides and methane. Transformation of the NOSH analytes was different in each of the hydrologic regimes, with the rate of transformation and the number of NOSHs degraded decreasing in the following order: drained > tidal > flooded. This can be clearly discerned in

Figures 10-12 with respect to the number of NOSHs transformed so as to afford a recovery of under 25 ppm at 16 weeks. Except for one sample showing a concentration spike at week 18, the drained system showed the elimination of quinoxaline, 2-methylquinoxaline, 2,3-dimenthylquinoxaline, and phenanthridine by week 16, with the recovered concentrations of the other NOSHs also diminished well below 25 ppm (i.e, tenfold concentration reductions). Under tidal conditions, quinoxaline, 2-methylquinoxaline, and phenothiazine were eliminated more rapidly than in any other treatment, with phenazine and phenanthridine reduced below 25 ppm by week 16. By 17.5 weeks, all NOSH analytes were transformed so that recoveries were less than 25 ppm. Under flooded conditions, only quinoxaline and dimethyl quinoxaline were degraded to recovery levels below 50 ppm within 16 weeks, and these were completely eliminated by week 17. Other than these, only phenothiazine and 2,3-dimethylquinoxaline were transformed to below 50 ppm recovery by week 20. The sediment concentrations of the other NOSHs remained static, with recovery levels remaining relatively constant between 100 and 170 ppm for acridine, phenanthridine, acridine, and phenazine between weeks 7 and 20. These results indicated that sediments and associated biota exposed to tidal pulsing (analogous to a streamside salt marsh ecophene) removed more NOSH compounds faster and to lower levels than a flooded sediment (analogous to a flooded interior ecophene or an impounded system). Although the drained sediments showed the fastest and most general degradation of the NOSH target analytes, this condition is not realistic in a "wetland" setting by definition: the sediments are not flooded, redox conditions are more characteristic of "uplands" and the vegetative situation would change within a few years because facultative wetland and upland species easily outcompete obligate wetland plants upon draining. Thus, of the two wetland possibilities (tidal vs. flooded), the tidal systems were better able to transform the NOSH compounds. From a contaminated sediment perpective, an optimal remediation stategy for these NOSHs would encompass dredging of affected areas followed by upland placement and tilling to maintain well-oxidized conditions. From an ecological perspective, promoting *in situ* remediation of NOSHs and other organic pollutants would involve exposing as much contaminated sediment as possible to the action of tides, while minimizing the area exposed to prolonged flooding. Obviously, this kind of undertaking in a real wetland would involve an integrated set of engineering interventions that encompass hydrologic, sedimentologic, and plant-system variables in a progressive sense. The author is aware of no actual cases in which this has been successfully attempted or contemplated: much further study is called for in ecological mesocosms and other controlled settings where important variables and true causal relationships can be identified and evaluated.

TABLE OF CONTENTS

LIST OF FIGURES

Introduction

The work discussed here addresses the influence of hydrology on biogeochemical processes that govern transport and transformation of selected pollutant chemicals in wetland sediment-plant systems. "Biogeochemical" refers to a group of coupled biological (e.g., microbial, plant) and physicochemical (e.g. flood-drain events from tides and floods) processes that to a large extent determine the productivity, habitat quality, and regional-global significance of wetland and other ecosystems (Catallo 1999; Catallo et al. 1999). Previous microcosm studies showed that degradation and transformation rates and pathways of aromatic hydrocarbons (AHs) and N-, O-, and S- heterocycles (NOSHs) differed significantly in marine sediments of different particle sizes and under oxidized vs. reduced conditions (Catallo 1996a, Catallo and Gambrell 1994). In these experiments, AH and NOSH transformation was evaluated in stirred, bubbled controlled Eh/pH reactors containing sediment slurries maintained as "oxidized, well-drained", "moderately reducing anoxic", and "highly reducing-methanogenic". AH and NOSH degradation rates generally were: oxidized ≥ moderately reducing >> methanogenic. The reactors employed for this work were first-order feedback control systems activated whose domains were predefined by acceptable Eh ranges for each treatment. As a result, Eh outputs were sinusoidal, and spectral analysis of the waveforms demonstrated that sampling interventions and other regular system perturbations were detected by the electrodes and preserved in the Eh vs. time record for each system. This suggested that the Eh might well change rapidly and reproducibly in response to other periodic forcing, such as diurnal tidal flushing, but this had not been examined in the previous literature. Further, it was clear that, among other difficulties discussed (loc. cit.), the terms "oxidized, well-drained" applied to wetland sediments constituted an oxymoron (by definition and by the fact that aggressive upland plants such as *Phragmites communis* soon displace obligate wetland plants upon draining of a normally flooded or tidal system). Nevertheless, this treatment type was evaluated because large quantities of wetland and aquatic sediments are disposed of in upland or otherwise well-drained situations, so evaluation of these sediments was deemed important, albeit not especially compelling from the perspective of "wetlands ecology".

Apart from the differential NOSH and AH transformation rates observed in these experiments, the results also suggested that the redox potential (Eh) as measured at Pt electrodes can be a dynamic nonlinear variable in wetland sediments that can respond quickly and significantly to changing conditions, and that this information can be preserved in time series of Eh provided adequate sampling intervals are employed. It was clear that the interaction of tides with sediments should be included as a treatment in further studies of biogeochemical processing of contaminants. In order to do this, much effort was expended in the design, monitoring, and evaluation of hydrodynamic sediment microcosms (20 gallon) and later, mesocosms (300 – 3000 gallons) containing a biocenosis comprised of sediments, plants, microbes, meiofauna, and macrofauna (Catallo 1999).

The central aims of the current study were: 1) determine the effects of static vs. dynamic hydrology on biogeochemical behavior of salt marsh sediments, particularly with respect to major output variables (e.g., Eh dynamics, trace gas signatures) and pollutant disposition and transformation, 2) design, build, and optimize microcosms for this purpose that are reproducible, well controlled, and adequately monitored in laboratory systems and, 3) develop

extraction/analytical approaches for NOSH target chemicals that allow for detection of small but significant concentration differences between time points and treatments. This last goal was especially important because most common methods used for identification of chemicals in complex mixtures are semi-quantitative (i.e., provide for order-of-magnitude differentiation in analyte concentrations), and it was expected that differences between NOSH transformation profiles in different treatments of this study would be below this threshold (Catallo 1996b).

The pollutant chemicals selected for this work included representative N-, O-, and S-heterocycles (NOSHs) that occur in coal chemicals, several crude oils, and pyrogenic (combustion-generated) mixtures (Figure 1; Turov et al. 1987). These and other NOSHs are found in pollutant mixtures near certain industrial activity, hazardous waste sites, and major harbors. They enter coastal marine and wetland ecosystems through a) direct dumping/spillage, b) fluvial transport of dissolved and sediment-associated chemicals from source outfalls, deposition of combustion-generated airborne particles, semivolatiles (e.g., from the so-called *in situ* burns of spilled oils) and polar residues and, c) assorted natural processes, including marine oil seeps and reactions between biogenic organic matter and sub- and super-critical water at deep sea hydrothermal vents. They are of continuing interest because they can accumulate to bioavailable levels in aquatic sediments and many NOSH compounds are acutely toxic, with some (e.g., the quinolines, carbazoles, and benzoquinolines and their oxidized metabolic and environmental transformation products) being mutagens and/or carcinogens in mammals (Catallo, 1996a, Warshawsky 1992, Catallo et al. 1994).

The target analytes initially envisaged for the sediment transformation work were quinoxaline, 2-methylquinoxaline, 2,3-dimethylquinoxaline, benzothiazole, dibenzofuran, phenazine, dibenzothiophene, phenothiazine, acridine, and phenanthridine (Figure 1). Unfortunately, synthetic and analytical problems arose with benzothiazole and dibenzofuran that were not tractable within the time frame of this work. Thus, these compounds were not examined and no data on their transformation in the context of this work is presented here. Other data, however, exist on the transformation of these materials in oxidized and reduced marine sediments of different particle size (Catallo, 1996a). The synthetic problems with these compounds were surmounted, and relevant data and references are provided herein which may be of use to future workers in these areas. Further, although large tidal mesocosms containing plants and macrofauna were developed, optimized, and examined at the end of the study presented here, expected continuance funding from MMS was not forthcoming, and these systems had to be dedicated to other work. As a result, chemical transformation results are available only from the tidal microcosms. A full description of these mesocosms can be found in the literature (Catallo 1999).

2

Quinoxaline   2-Methylquinoxaline   2,3-Dimethylquinoxaline

Dibenzofuran   Phenazine   Dibenzothiophene

Phenothiazine   Acridine   Phenanthridine
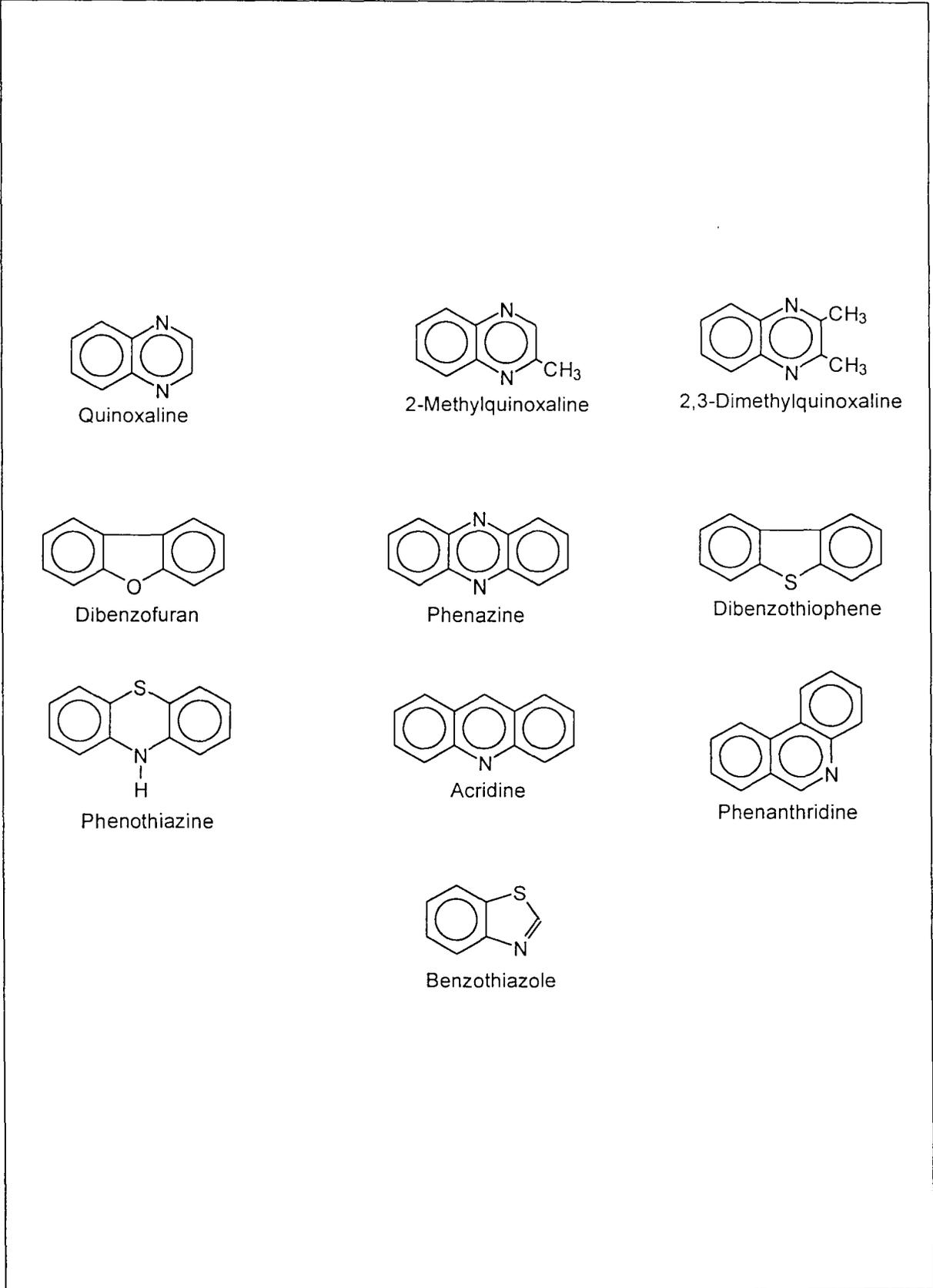
Benzothiazole

Figure 1. NOSH Target Analytes Examined in This Study

Materials and Methods

The main areas of research and development for this study were 1) hydrodynamic ecological mesocosm design, maintenance, and characterization, 2) process output signal detection (sensors, electronics, EPROM and other low level software), acquisition (portable, multi-channel data acquisition), and analysis (wavelet transform and signal reconstruction) and, 3) synthetic organic and environmental analytical chemistry (developing ways to detect subtle differences in chemical transformation between treatments using available instrumentation). These will be summarized below.

Hydrodynamic Wetland Microcosms. These systems were comprised of twenty (20) gallon glass aquaria containing salt marsh sediment columns (Figure 2). Salt marsh sediments were collected from Terrebonne LA, mixed with sand and commercial seed starter (5% w/w, each) and wetted with sterile Instant Ocean (15 ppt). The sediments then were added to cylinders (26 cm i.d.) made first of wire, and ultimately of plastic mesh (0.5 cm) to a height of 38 cm. The wire mesh was discontinued after trial runs because it interfered with Eh measurements. The systems were covered with plexiglass containing throughputs for gas sampling and electrode wire leads. A diurnal tide was simulated by pumping artificial seawater (15 g/L Instant Ocean) into and out of the aquarium once daily (i.e. one high and one low per 24 hours) using peristaltic pumps and controlled by battery-powered timers. The seawater resevoir was continuously aerated. At "high tide", the sediment columns were covered with ca. 8 cm of water. At "low tide" about 5 cm of water remained in the aquarium. Two Eh electrodes (below) were positioned in the top 1 cm of sediment (surface) and two additional electrodes were placed deep within the column, in the continually flooded zone near the bottom. Trace gases were analyzed by direct sampling of headspace gases under vacuum to a 10 meter cell connected to a Buck Scientific FTIR spectrometer with gold optics and 4 cm^{-1} resolution (Figure 3). Molecular sieve 5A and Ascarite A (Aldrich) traps were used to reduce water and CO_2 spectral interference and increase sensitivity. Compounds were identified using authentic gas standards (Scott Specialty Gases) and published spectral libraries from NIST. Digital background subtractions were performed using spectra of normal laboratory air sampled immediately prior to sampling the microcoss, and handled identically (i.e., reference air samples were passed through the sampling loop of the system and through the traps). Thus, CO_2 and water vapor signals in the experimental spectra represent excesses of these materials in the microcosm headspaces vs. the laboratory air.

Redox potential was logged using Pt wire –SCE electrode cells and a multichannel logger (Catallo 1999 - below). The platinum electrodes were constructed by welding Cu wire to 0.5 cm lengths of 12 guage Pt wire. The junction and Cu lead were enclosed in glass tubes of varying lengths, and both ends were sealed using a blowtorch. Hence, a ca. 0.5 cm length of Pt was exposed to the sediments while junctions and leads were enclosed in sealed glass. The electrodes were polished with light emory paper, soaked in aqua regia and then distilled water. Each electrode was calibrated prior to use with a 1% quinhydrone solution.

The fidelity and response time of potenial measurements was examined by connecting the Pt end of each electrode to the working lead of a EG&G PAR 173 scanning Potentiostat and sweeping the potential positive and negative between +1 and -1V vs. the saturated calomel electrode (SCE). The potential registered by the Pt electrode was monitored using a voltmeter attached to the Cu lead. The purpose of the calibration and response tests was to confirm that the
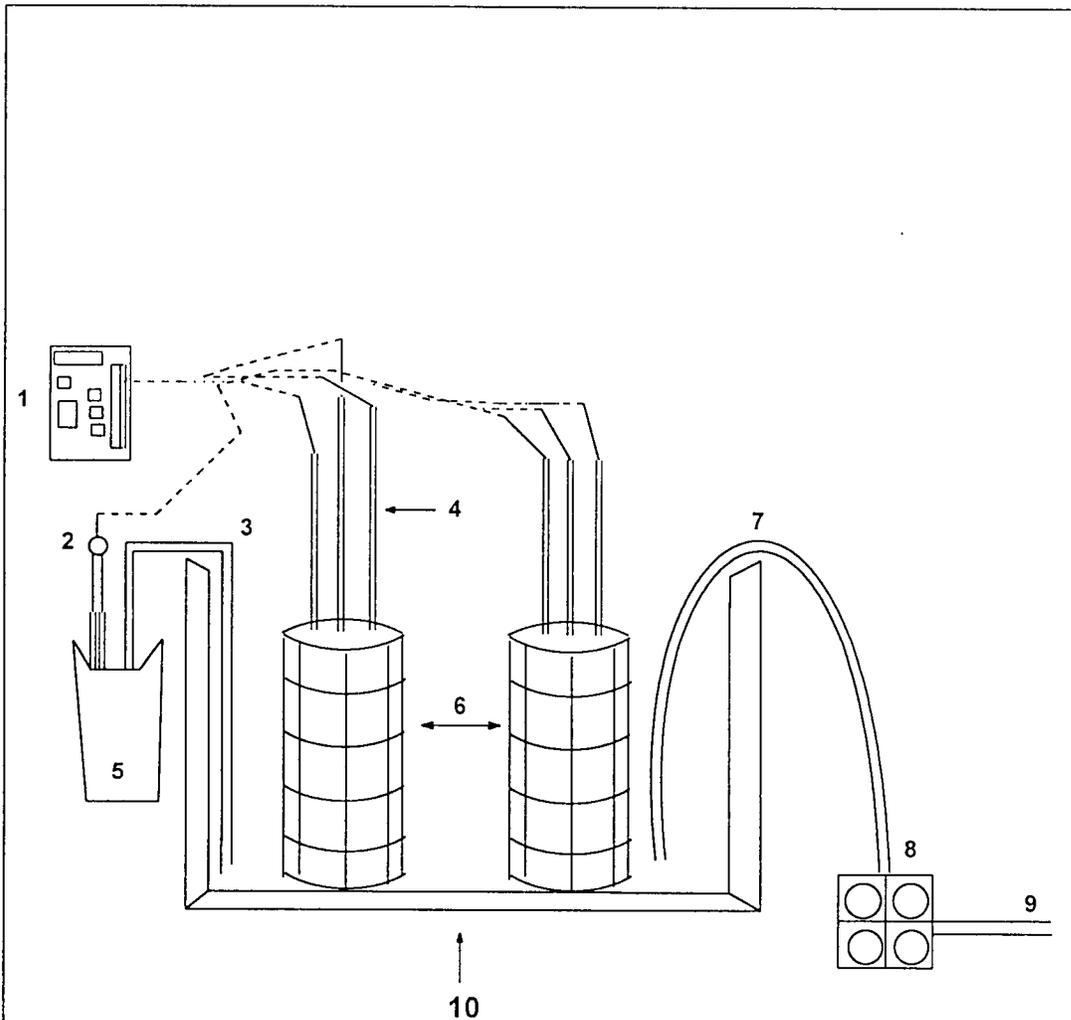
Schematic Diagram of Tide Simulation Microcosm. 1. Data logger, 2. calomel reference electrode, 3. salt bridge, 4. platinum electrodes, 5. concentrated KCl solution, 6. soil column enclosures, 7. water inlet/outlet, 8. automatic peristaltic pumps, 9. resevoir feed.

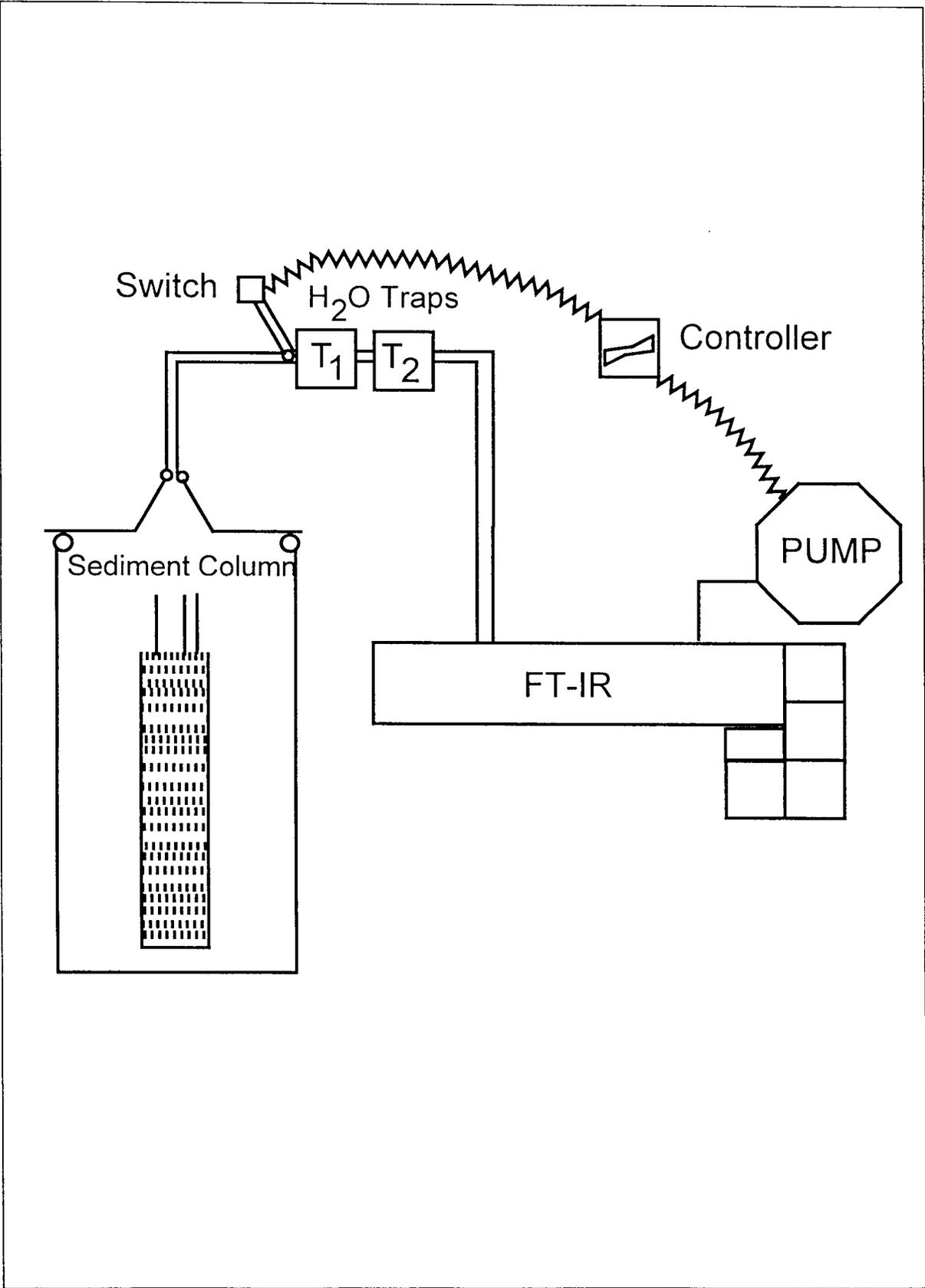Figure 2. Schematic Diagram of the Tide Simulation Microcosm

Figure 3. Schematic Diagram of the Gas Sampling/Analysis System

electrodes provided accurate solution Eh readings, and that these readings were instantaneous and within 5 mV of one another (i.e., there were no changes in junction potentials or other interference within the experimental electrodes). Electrodes failing the quinhydrone calibration or not accurately and rapidly registering positive and negative potenial changes generated by the potentiostat were rejected.

Eh data were logged using a custom, stand-alone data acqusition-storage module developed and constructed in the laboratory. The data logger was designed to be field deployable and replace a large data acquisition system (National Instruments MIO-16 acquisition board, SCXI preamplifier/multiplexer, 486/66 microcomputer with LabView Software) in experimental situations where available space or portability were of issue. The data logger was constructed with the following features: a) electronic components have ultra-low standby current requirements (1.3 μA total system in standby mode, 30 μA during acquisition sweep of 1 ms); b) the components are stable to environmental temperatures (-40 oC - +70 oC); c) total memory 32 kbytes and data acquisition rates are programmable 1 min - 24 h. Data can be downloaded in situ via RS232C serial port and IBM compatible computer (including notebooks and portables). Each unit consists of 12.5 x 10.5 cm board, seven input channels with single or individual reference capabilities (continuous monitoring of a 2.342 V reference). The unit was powered by battery (5.5 - 12 V), but a solar panel or normal AC/step-down transformer sources also could be used. The manufacturing cost of the system was low (with printed circuit boards around $15) so the unit was considered expendible if lost/destroyed during field work. With a standard 6V battery and a sampling interval of 1 h, the unit could stand alone in the field for approximately 100 d. Samples were collected at rates between 1 Hz and 1/h, depending on the application (in general, the rates were 1-2/h).

Hydrodynamic Wetland Mesocosms. The few available sources on construction and theory of microcosmology (e.g., Beyers and Odum 1993), provide very little practical insight to the construction and behavior of hydrodynamic wetland mesocosms. The "tidal" systems one finds are oriented to small scales with, in one case water reservoirs moved vertically using timed stepper motors (Beyers and Odum 1993). This may be practicable at small scales (e.g., 20 gallons), but if one desires to evaluate true ecological volumes (e.g., > 10 times this size) encompassing plants and perhaps macrofauna as well as sediments, microbes and water, then an entirely different approach would seem to be called for. The basic design ethos used here was to keep moving parts to a minimum, strive for simplicity so that failure modes would be relatively limited, constrain costs, and use available, easily replaceable (mass-produced, modular) components. The mesocosm that evolved had a volume of 300 gallons, but the basic design was easily upscaled to 10 times that size or larger (Figure 4). Basically, each system relied on only one machine (a pump), and its function was controlled by a binary (on-off) timer. The pump moves water out of the mesocosm into elevated storage tanks over a 12 h period simulating a falling tide. When the storage tanks are filled this activates a siphon which returns the water to the mesocosm with simple flow restriction used to control the return time (again, 12 h). The other managed input was artificial sunlight, which shone for 10 hours per day followed by 14 of relative darkness. The lights were synchronized with normal day time because the mesocosms were located in a laboratory annex containing no climate control and a large wall fan open to the outside environment. Sediments and plants (smooth cordgrass or oyster grass, *Spartina alterniflora*) were collected from Louisiana salt marshes and were replaced with our greenhouse-

1. Light banks with timers, 2. Plants, 3. Sediment,
4. Agricultural mesh and burlap, 5. Oyster Shell,
6. Pumps, 7. Cobble Stones, 8. Reservoirs,
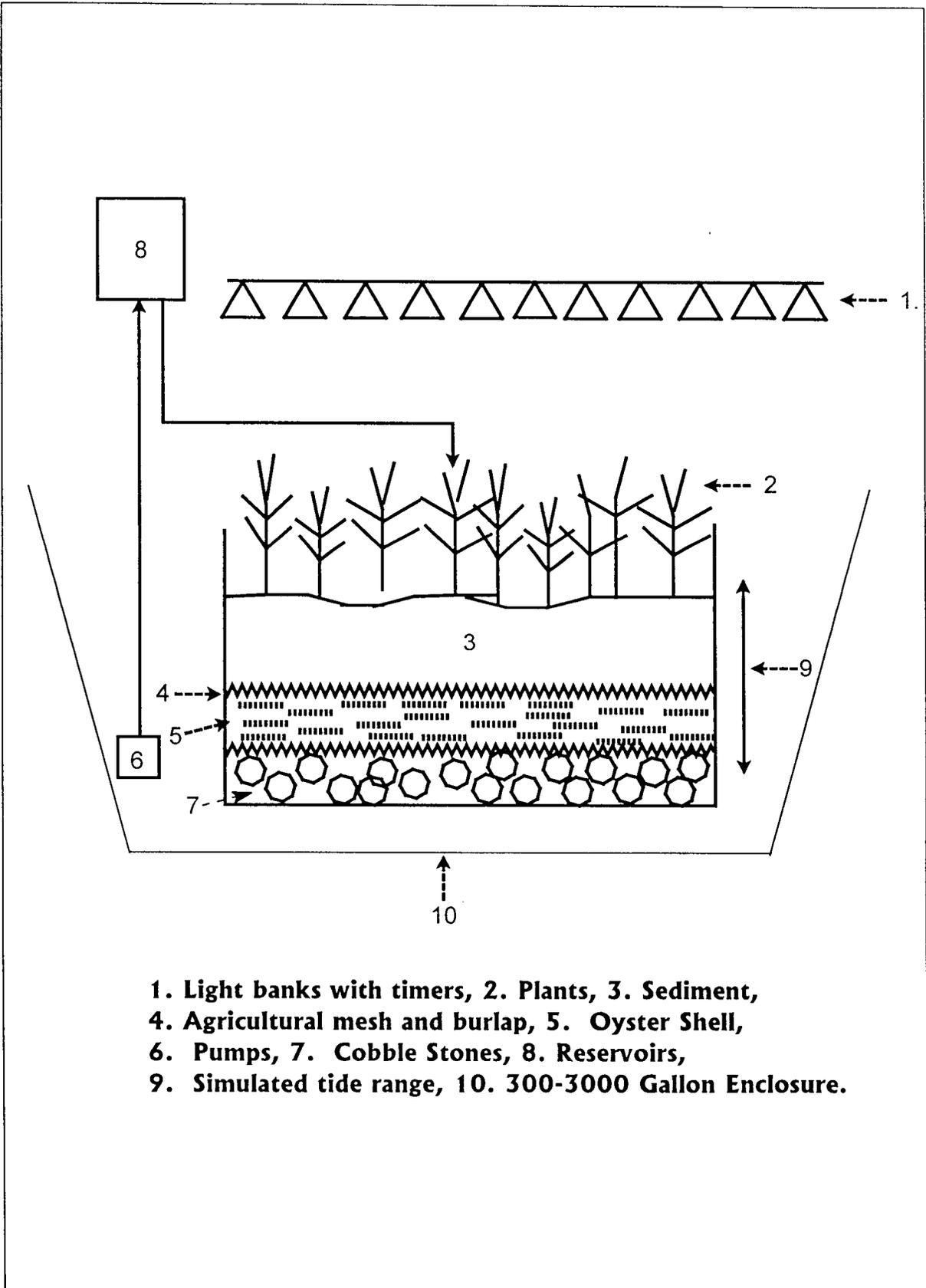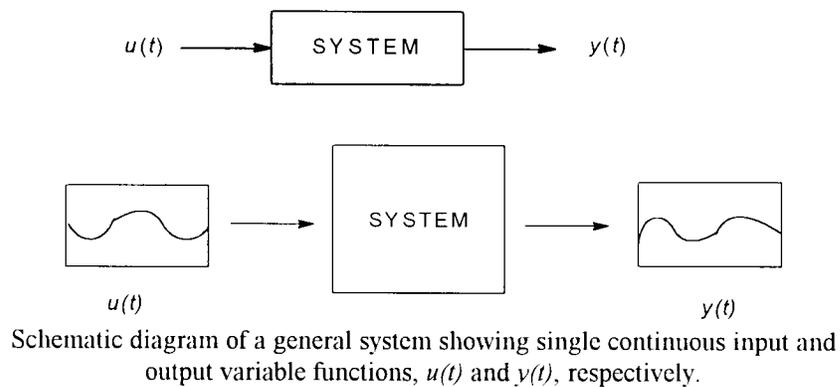9. Simulated tide range, 10. 300-3000 Gallon Enclosure.

Figure 4. Schematic Diagram of the Tidal Wetland Mesocosm

reared *S. alterniflora*. Only this dominant plant was collected: no attempt was made to reproduce the vegetative diversity of the marsh by collecting the other typical salt marsh species. The plants were transplanted to six 300 gallon mesocosms which comprised duplicates of each hydrologic condition to be examined: *flooded-anaerobic* (oxygen deficient), with water exchanged weekly, *drained-aerobic* (well oxygenated), with water flushed into the system weekly, and 3) *tidal*, pulsed by a simulated diurnal tide. Data indicated that the tidal sediments alternated between mildly anaerobic and aerobic during the course of the of the flood drain cycle. The "ocean water" used was a commercial material of identical elemental composition as seawater, and this was inoculated with authentic estuarine water at about 20% by volume. These systems have been run for as long as two years, and the annual grass *S. alterniflora* was observed to go through its entire life cycle including producing viable seeds and maintaining some known fungal (Catallo et al. 1996c) and insect endosymbionts.

Signal Acquisition and Analysis. A biogeochemical system can be viewed as a signal processor, with inputs (e.g., light and heat cycles, water fluctuations, inputs of organic matter and toxic chemicals) impinging on the system, influencing its behavior (e.g., aerobic vs. anaerobic microbial metabolism), and influencing the magnitude and time structure of numerous outputs (water chemistry; trace gas identities and evolution time series, below). The inputs

Schematic diagram of a general system showing single continuous input and output variable functions, *u(t)* and *y(t)*, respectively.

mentioned are impulses or excitation functions having importance to system processes *and all of these must be known (identified) and measured accurately with appropriate sampling frequencies*. The former is important because confounding and collinear variables can invalidate normal statistical approaches and corrupt causal ascriptions (Catallo et al 1999; Asher 1983) and the latter because processes changing faster than the sampling rate applied to them can cause error (aliasing) when spectral analysis techniques are applied to the time series (Brockwell and Davis 1991; Mallat 1998). These functions, ($u(t)$), can be of many kinds: e.g., linear, spike, ramp, threshold, sinusoidal. Signals are outputs, $y(t)$ carrying information about system processes. Signals are structured in time, whereas noise is not, and increase relative to noise as the square root of the sampling repetitions; $(S/N)_n = n^{1/2}(S/N)_1$; where S is signal amplitude, N is noise amplitude, $(S/N)_1$ is signal:noise ratio after one data acquisition (scan), n is the number of sample scans, and $(S/N)_n$ is the signal:noise ratio after n scans. The states of the system can be defined by the stable and transient domains occupied by output signals $y(t)$.

It frequently is assumed that biogeochemical processes change slowly (i.e.,weeks-months) and therefore adequate sampling of many outputs can be performed at rates on the order of 1/day

to 1/month (Catallo 1999). Unfortunately, it must first be verified that there are no cyclic outputs from the system with frequencies greater than the sampling rate, and this is rarely done. As a result, the assumption that widely-spaced data points from dynamic systems can be connected by linear interpolation to accurately reconstruct system temporal behavior becomes a matter of faith rather than demonstration. Further, attempts to analyze such a time series in order to determine its component waves can be highly inaccurate and misleading. The upshot of this is that complex system variables ($u(t)$) and signals ($y(t)$) cannot be interpreted accurately if data points are gathered rates slower than the changes in the process. For virtually all ecological system processes, classical single resolution Fourier analysis of time series suffers from major analytical drawbacks, not the least of which being its inability to accommodate nonstationarity in the the data -i.e., transients and other deviations from perfect statistical regularity in the signals. Single resolution analysis of nonstationary time series leads to aliasing of power spectra (Brockwell and Davis 1991; Mallat 1998) in ways that are not readily interpreted or identified. It was clear from preliminary time series of Eh, pH, temperature , and humidity on the microcosms that nonstationarity was a major feature of the input and output variables of concern in this work. As a result, multi-resolution "wavelet" analysis was applied to the time series of signals from the microcosms.

Wavelet analysis is a recent development in applied mathematics (Mallat 1998) that has stimulated an enormous interest in many disciplines including signal processing/reconstruction, vision science, medical imaging, and physiology. The wavelet transform is a technique that allows for the acquisition of both localized time and frequency information about a signal. It's ability to dilate and shift time windows with respect to a signal allows for transient features to be accommodated, long and short term variations to be compensated, and periodic features to be isolated, identified, and if needed, enhanced. In a sense, the wavelet approach has operational and conceptual affinities with the use of an oscilloscope to visualize waveforms in circuits: time sampling windows are varied by the analyst in an attempt to isolate component waveforms of complex signals as standing waves, with frequency data available as the inverse of the time window used to capture the particular wave of interest. Waveform components and their alterations by specific processors are obtained by thoroughly permuting the available sampling windows.

The definition of the continuous time wavelet transform (CWT) entails an "analysis wavelet," ψ (t), and the family of shifted and dilated versions

$$\psi_{a,b} = |a|^{-1/2} \psi (t-b)/a$$

The transform of x (t) $\in L_2(R)$ is then

$$T^w[x] (a, b) = |a|^{-1/2} \int x(t) P^*(t-b)/a\, dt$$

The analysis wavelet, ψ must satisfy the admissibility condition

$$C_p = \int |\Psi(w)|^2 /w\, dw < \infty$$

This condition guarantees the existence of the inverse transform

11

$$x = C\psi^{-1} \int \int T^w [x] \, (a,b) \, \psi \, *(t - b)/a \; dadb; \text{ (integrals evaluated } \pm \infty)$$

One way to realize the transform is using the filter bank interpretation and defining

$$h_a \, (t) \; = \; \psi \, (-t/a)$$

It follows that

$$T^w [x] \, (a,b) = x \, (t) \, h_a \, (t) \; = \int x(t) \, h_a \, (b - t) dt$$

So, for each scale, a, the CWT is comprised by the output of a filter with impulse- response $h_a \, (t)$. Since the filters are dilated according to a scale parameter, their frequency response is modified accordingly, and with the discretion of the analyst. Hence, the wavelet transform maps a function of one variable into a function of two independent variables (time, scale). There is, as a consequence, significant redundancy and if desired, the time/scale parameters can be rendered discrete and the signal will still be preserved in the transform samples. A standard discretization approach employs a logarithmic step for the scale parameter and a scale-dependent discretization for the temporal parameter. Thus a discretized wavelet transform can be defined via

$$T^{w[m,n]} = T^w[x] \, (2^m, 2^m n)$$

In effect, a family of wavelets of the form $\psi_{m,n} = 2^{-m/2} \psi(t \, / \, 2^m - n)$ is deployed.

Even in the discretized case the representation can be optimized by requiring the wavelet family, $\psi_{m,n}$ to be an orthonormal as a set. In this case for each value of the integer m, the collection of vectors $\{ \psi_{m,n}; \, n \in Z \}$, defines a subspace, W_m. As the dilation parameter is held constant, all wavelets in that subspace have the same scale. The projection of a function, $x(t)$, on this subspace is interpreted as the details of the function at that particular scale. Since increasing the value of the integer, m, introduces larger dilations in the basic wavelet, its time resolution decreases. The sum of all details for all values $m \geq m_0$ is said to give a representation of $x(t)$ up to resolution m_0. Mathematically, the representations exist in the subspace

$$V_m = \oplus_{k \geq m} W_k$$

One of the bulwarks of multiresolution analysis is that a unique analysis function $\varphi \, (t)$ can be defined such that the collection $\{ \varphi_{m,n} \; = 2^{-m/2} \psi(t \, / \, 2^m - n) \}$ spans the subspace, V_m, and these are easy to compute recursively. If, for example the representation of a signal up to a fine resolution level, m_0, is in the form

$$X^{mo} = \sum_n c^{mo}_n \, \varphi_{mo,n}$$

one can define a digital filter, H, receiving as input the discrete signal, $\{ \; c^{mo} = c^{mo}_n; \, n \in Z \}$ and producing as output the coefficients of the next lower resolution, c^{mo+1}. Similarly, the digital

filter, G, can be defined as receiving the same input and producing as output the details at resolution level m_0.

The wavelet packet approach applies to discrete time functions, such as those obtained by sampling a continuous time signal. It can be considered as the discrete version of the multiresolution decomposition. Using suitable digital filter banks, a discrete time signal is decomposed into orthogonal components which also have disjoint frequency changes. Then, by combining various orthogonal components one can develop representations of the signal at various resolution levels. For cases of ecological signals, such as the Eh time series realized in the tidal microcosms of the current study, periodic features, transients, and long term trends can be determined without unknown aliasing in resulting spectra. A further advantage is that the effects of low resolution sampling can be examined, and the resulting effects of aliasing on the signal analysis evaluated. This also was applied to the Eh time series as an illustration of the perils of under-sampling outputs dynamic systems and then applying spectral analytical or other analytical techniques to those data.

Synthetic Organic Chemistry. In order to quantify minor or subtle differences in transformation of target compounds between hydrological/biogeochemical treatments, it is desirable to have stable isotope-labeled (e.g., deuterated) standards for each target compound of interest. These standards can be added to sediments before extraction, and serve as internal "monitors" of extraction and analytical efficiencies for the target compounds. As these deuterated standards have properties virtually identical to the target compounds, their loss in the various steps of extraction and analysis are the same as the compounds under study. With appropriate controls and other procedures, this allows for quantitative analyses (percent differences can be detected), rather than semi-quantitative data (order-of-magnitude differences are detected) provided by many analytical approaches (Catallo 1996b). In addition to the quantitative nature of isotopic dilution vs. the order-of-magnitude or semi-quantitative nature of standard GC-MS of environmental extracts, the deuterated standards also allowed for data streamlining: when isotopic dilution standards are used in known amounts, quantitation is simply a matter of integral ratios between the standard (known concentration and base peak integral) and the target analyte (concentration unknown, base peak integral known). Unfortunately, commercial availability of all NOSHs is limited or nonexistent. As a result, methods were developed in our laboratory to label the target compounds with deuterium either using *de novo* or post-synthetic approaches (reviewed in Junk and Catallo 1996), the latter typically using supercritical deuterium oxide (D_2O), or "heavy water". A basic example of this strategy is given below:

13
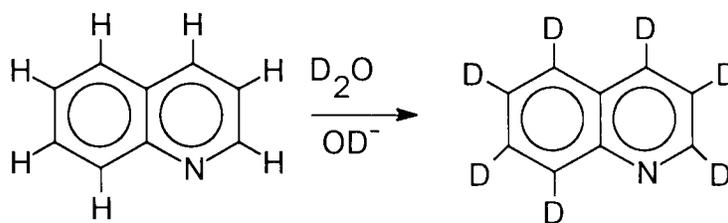
Supercritical water is extremely corrosive, and commercial stainless steel reactors leaked or exploded (destroying furnaces and part of a wall) after a few hours. As a result, a substantial amount of effort in the first year of this work was devoted to designing reactors that withstand supercritical aqueous conditions and were large enough to permit multi-gram labeling of target compounds. With this accomplished, the target compounds were made in quantities sufficient for use in the transformation studies of NOSHs in the microcosms. Unfortunately, NOSHs including benzothiazole and phenothiazine were unstable even in subcritical water, and novel synthetic protocols had to be devised for them.

Benzothiazole-d1 was prepared according to a published procedure found in Chikashita et al. (1989). It was found that some H-D exchange occurred when the deuterated material was exposed to water over several months. Further, the pure compound was clear, and storage over several months gave rise to a burnt red color. The pure compound then was obtained by distillation. Benzothiazole(4,5,6,7)-d4 was synthesized according to a synthesis developed for this study and detailed in Junk et al. 1997a.

Quinoxaline-d6, 2-methylquinoxaline-d8, and 2,3-dimethylquinoxaline-d10 also were prepared by syntheses developed for this work and published in Junk et al. 1997b.

Attempts to prepare phenazine-d8 and acridine-d9 by a) base-catalyzed supercritical isotope exchange at 400 °C for 6 hrs following Junk and Catallo (1996, 1997), b) acid catalyzed isotope exchange at 220 °C for two days, and c) palladium catalyzed exchange at 250 °C for two days all resulted in extensive substrate decomposition. All compounds were subsequently prepared by base-catalyzed near-critical exchange at 300 °C for four hours. Exchange was carried out by heating 300 mg substrate, 15 mL D_2O and 0.1 mL 40% NaOD (in D_2O) in a 30 mL Hastelloy C-22 autoclave. The crude products were extracted with dichloromethane, the solvent evaporated and deuterated quinoxaline and 2,3-dimethylquinoxaline purified with a microdistillation apparatus. Phenazine and acridine were crystallized from methanol. Further purification was achieved by chromatography (200 mesh silica gel, hexane:DCM 10:1 v/v). Yields ranged from 45 to 55%.

Dibenzofuran-d8, dibenzothiophene-d8, phenothiazine-d8, phenanthridine-d9 were prepared using the published Supercritical Isotope Exchange (SDE) technique of Junk and Catallo (1996), which has been awarded a US Patent (Catallo and Junk 1998). A 30 mL Hastelloy-C22 autoclave was charged with the 0.3 g of the respective substrates, 15 mL deuterium oxide, and 0.1 mL 40% deuterium deuteroxide solution. Exchange was achieved by heating to 400 °C for 6 hrs. Compounds were collected by filtration and purified by chromatography over 200 mesh silica gel using hexane as mobile phase for dibenzofuran, dibenzothiophene, and phenanthridine, dichloromethane for phenothiazine. Yields for dibenzothiophene and phenanthridine were above 75% and have been reported along with a range of other AHs and NOSHs (Junk and Catallo 1996). Those of dibenzofuran and phenothiazine were 92 and 86%, respectively. No attempts were made to preclude the facile back-exchange of the N-H proton of phenothiazine during workup under ambient (open-air) conditions because this analyte would be exposed to water during the sample extraction.

NOSH Transformation Studies. Three sediment columns were equilibrated under drained, flooded and tidal conditions for two weeks. The sediments then were collected from the surface third of the enclosures from each hydrologic type. Protiated (hydrogenated) NOSH compounds in acetone were added to the sediments with mechanical mixing (2 h) to provide uniform levels of

the individual target contaminants between 200 and 400 ppm on a weight basis. Care was taken to maintain the biogeochemical condition of the sediments (oxidized vs. reduced) during mixing by purging the system and flooding the headspace of the mixing enclosure with Ar. The sediments then were reintroduced to their respective microcosms in a fresh column enclosure. The three hydrologic regimes were initiated and "time zero" samples were collected within 8 h of placement using a glass corer. Subsequent samples were taken at intervals (1-4 weeks) depending on estimators of microbial activity and previous recovery of NOSH target analytes. After collection, the sediment-water samples (ca. 10 g) were weighed and amended with deuterated isotopic dilution standards for each NOSH target analyte at the levels near the sediment concentrations. The samples then were Soxhlet extracted with dichloromethane (DCM - 48 h), with the extract subsequently dried (N_a2SO_4) and concentrated under N_2. The residual sediments in the extraction thimbles were dried and weighed. The sample extracts then were subjected to GC-MS in the full scan mode. NOSH target analyte concentrations in the extract were determined vs. the isotopic dilution standard by ratios of correpsonding peak integrals. These extract concentrations were corrected for concentration factor and dry weight of sediment in the original sample. An example of the resolution of protiated NOSH analytes and their respective deuterated isotopic dilution standards is given in Figure 5.
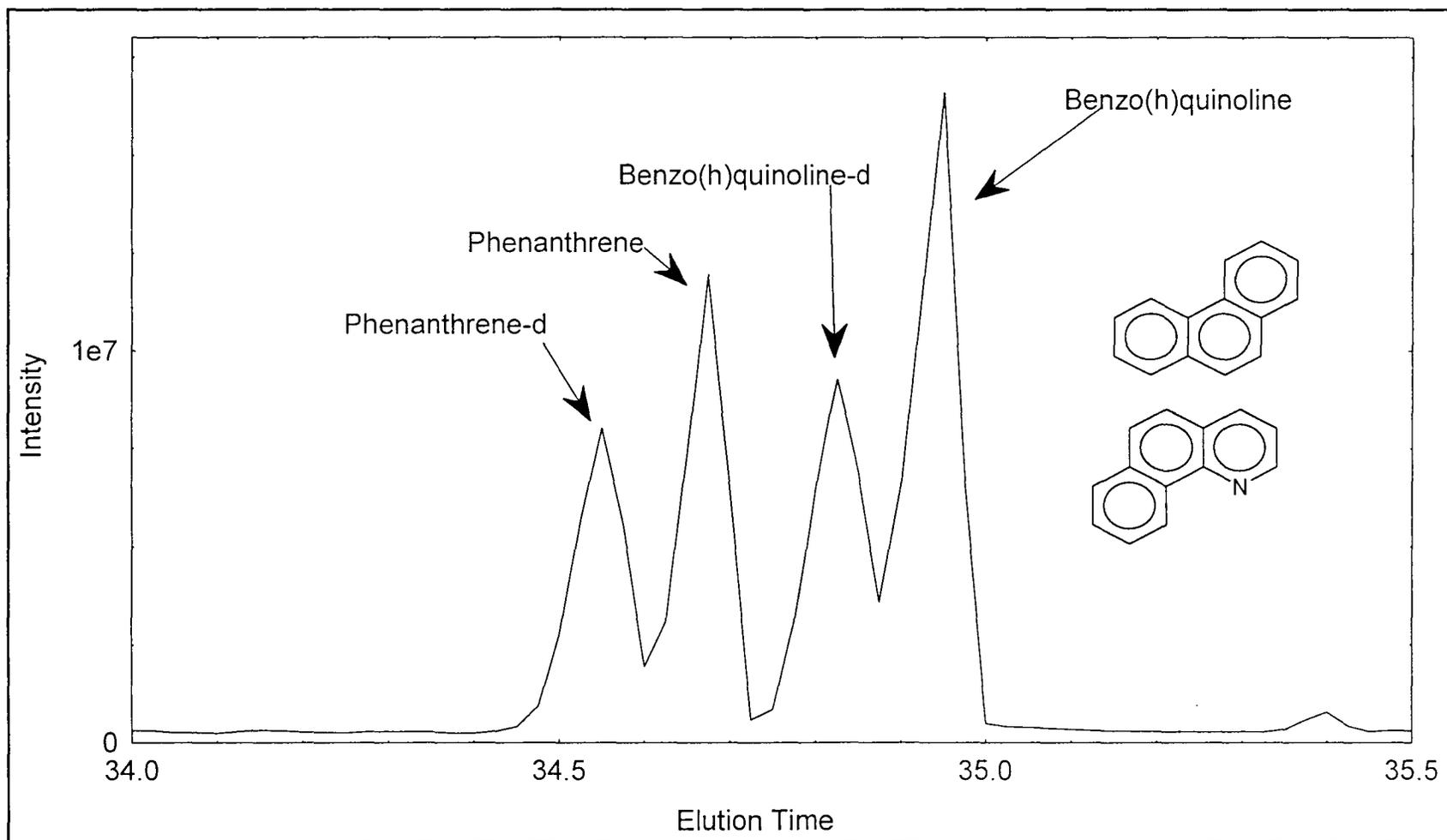
Figure 5. Total Ion Trace of Protiated NOSH Analytes and Deuterated Isotopic Dilution Standards

Results and Discussion

The biogeochemical behavior of the hydrodynamic microcoms was very similar to that observed in natural settings, and in accord with prevailing theory (Odum 1983, Catallo 1999). Redox potential time series and sediment trace gas signatures from the different treatments were analogous to those encountered in field settings (Catallo 1999). With respect to the redox potential, the continuously flooded and drained systems had reduced (mean - 428 mV) and oxidized (+ 73 mV) Eh values, respectively, with no evidence of daily or longer periodic variation. The routine (weekly) wetting/draining of the drained system, and water changing in the flooded system did not affect these trends: apparently the pre-equilibration of sediments prior to addition of the NOSH analytes afforded a degree of redox potential poising (i.e., "buffering") that was not affected by the brief maintenance interventions. The tidal systems, however, exhibited oscillating Eh values, with significant amplitudes (40 – 180 mV; Figure 6). Analysis of theses signals using the wavelet transform confirmed the presence of strong diurnal Eh variations, with a mean value period of 23.78 ± 2.10 h. It has been shown that these diurnal signals were 1) reproducible in the tide microcosms, 2) found in the corresponding tidal mesocosms, which also contained the plant *S. alterniflora* and, 3) observable in tidal field sites vs. impounded areas not receiving tidal input (Catallo 1999). Thermal and light cycles in the laboratory had no discernable effect on the Eh time signature: variation of tide stage throughout the day and at different times of the year (the experiments were conducted in an annex not equipped with climate control) did not affect the tide-derived Eh signal period.

As an exercise, the effect of sampling frequency on the detection and accurate isolation of the 24 h waveform in the Eh data was examined by simulating the time series derived from a 16 h sampling frequency (not uncommon in laboratory studies, and much more rapid than most field studies). The results were striking: sampling at 1/16 h eliminated the detection of the 24h waveform in the Eh time series, and subsequent wavelet analysis gave the (erroneous) result of a waveform close to 48 h in period.

The Eh traces shown in Figure 6 are characteristic of the tidal systems in three main respects: the sinusoidal Eh oscillations continued for as long as the tides were applied, but ceased immediately when static conditions ensued, 2) the waveforms are asymmetric, exhibiting hysteresis with respect to oxidation and reduction and, 3) electrodes at the different depths were out of phase (as expected), with deeper electrodes showing smaller amplitudes (probably reflecting the effects of compaction). These features were consistent through the experimental runs, and with subsequent work (Catallo 1999). The hysteresis of measured redox potentials (item 2, above) suggests that the Pt electrode-Ag/AgCl cell is quasi-reversible with respect to oxidation and reduction in sediments (i.e., it is patently non-Nernstian) and this almost certainly reflects compositional as well as bulk phase chemical variables *in situ*. Unpacking these factors and adequately describing the physics involved is far beyond the scope of this communication, and might well be impossible given the current level of theory on heterogeneous electrochemical systems. In spite of this problem, the electrodes in the tidal systems remained functional throughout the duration of the experiments, and rarely had to be replaced. The electrodes in the oxidized and reduced static microcosms, however, were replaced frequently because of passivation with oxides and sulfides (respectively). It would seem that dynamic Eh conditions reduced the level of passivation on the working electrodes, and provided for conditions most favorable for obtaining accurate potential values vs. the more static, well-poised conditions in the
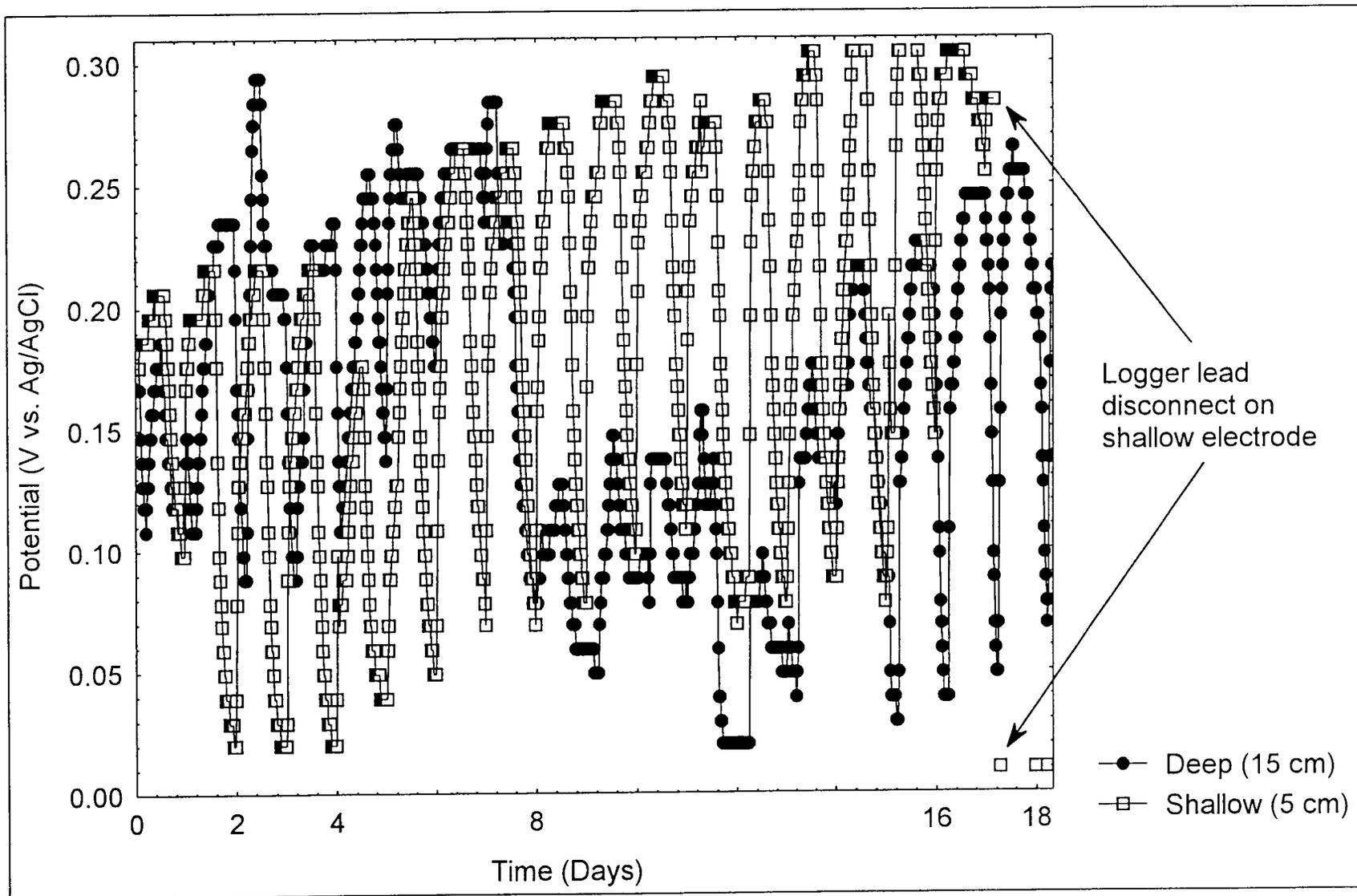
Figure 6. Time Series of Eh from Shallow and Deep Electrodes Placed in the Tide Simulation Microcosms

other treatments. "Reconditioning" of solid electrode surfaces by alternating oxidation-reduction cycles (i.e., cyclic removal of surface metal oxides and sulfides by alternating redox processes) has not been reported elsewhere, and represents an area of interest for further research.

Biogeochemical gas mixtures leaving the sediments also reflected the hydrological status of the treatments: the major gases observed were CO_2 from microbial respiration (drained, Figure 7), vs. CO_2 with small methane signal (tidal; Figure 8), vs. large amounts of methane (flooded conditions; Figure 9). The gas profiles in each treatment were checked weekly, and were consistent throughout the experiment. The FTIR analysis of trace gases used here was adequate for crude monitoring purposes, but factors including spectral interferences (e.g., H_2O and H_2S; CH_4 and $(CH_3)_2S$), low sensitivity of detection for some sulfides (e.g., H_2S), and adsorption of volatile hydrocarbons and sulfides on the traps makes this approach untenable for high resolution work. This is why "sulfides + methane" is indicated on some figures: sulfides could be smelled during these sampling interventions (and on the traps afterwards), and the methane resonances around 1300 cm^{-1} clearly were augmented by sulfides, most likely mixtures of H_2S and dimethylsulfide. As a result of this difficulty, intermediate resolution (2 - 6 cm^{-1}) FTIR is not well-suited to detailed evaluation of biogeochemical gas mixtures from sediments, but can be used for low resolution "spot checks" and single analyte monitoring in cases where one gas clearly predominates in a mixture and can be unambiguously detected. More specific approaches including photoacoustic FTIR and headspace GC-MS offer increased sensitivity, very high resolution, and accurate 3-dimensional identification of sediment gases, but these approaches have yet to be evaluated in the current context.

Transformation of the NOSH analytes was different in each of the hydrologic regimes, with the rate of transformation and the number of NOSHs degraded decreasing in the following order: drained > tidal > flooded. This can be clearly discerned in Figures 10-12 with respect to the number of NOSHs transformed so as to afford a recovery of under 50 ppm at 16 weeks. Except for one sample showing a concentration spike at week 18, the drained system showed the most complete elimination of quinoxaline, 2-methylquinoxaline, 2,3-dimenthylquinoxaline, and phenanthridine by week 16, with the recovered concentrations of the other NOSHs also diminished to or below 25 ppm (i.e., a tenfold concentration reduction). Under tidal conditions, quinoxaline, 2-methylquinoxaline, and phenothiazine were eliminated more rapidly than in any other treatment, with phenazine and phenanthridine reduced below 25 ppm by week 16. By 17.5 weeks, all NOSH analytes were transformed so that recoveries were less than 25 ppm. Under flooded conditions, only quinoxaline and dimethyl quinoxaline were degraded to recovery levels below 50 ppm within 16 weeks, and these were completely eliminated by week 17. Other than these, only phenothiazine and 2,3-dimethylquinoxaline were transformed to below 50 ppm recovery by week 20. The sediment concentrations of the other NOSHs remained static, with recovery levels remaining relatively constant between 100 and 170 ppm for acridine, phenanthridine, acridine, and phenazine between weeks 7 and 20. It would seem then, that except for quinoxaline, 2-methylquinoxaline, and perhaps phenothiazine, there was a redox effect on transformation rate for the NOSH compounds studied here.

These transformation studies, while preliminary, clearly indicated that tidal flushing optimizes the transformation of some NOSH compounds, and that in situ or "passive" remdiation of coal- and petro- chemical pollution in coastal wetlands should include design features that
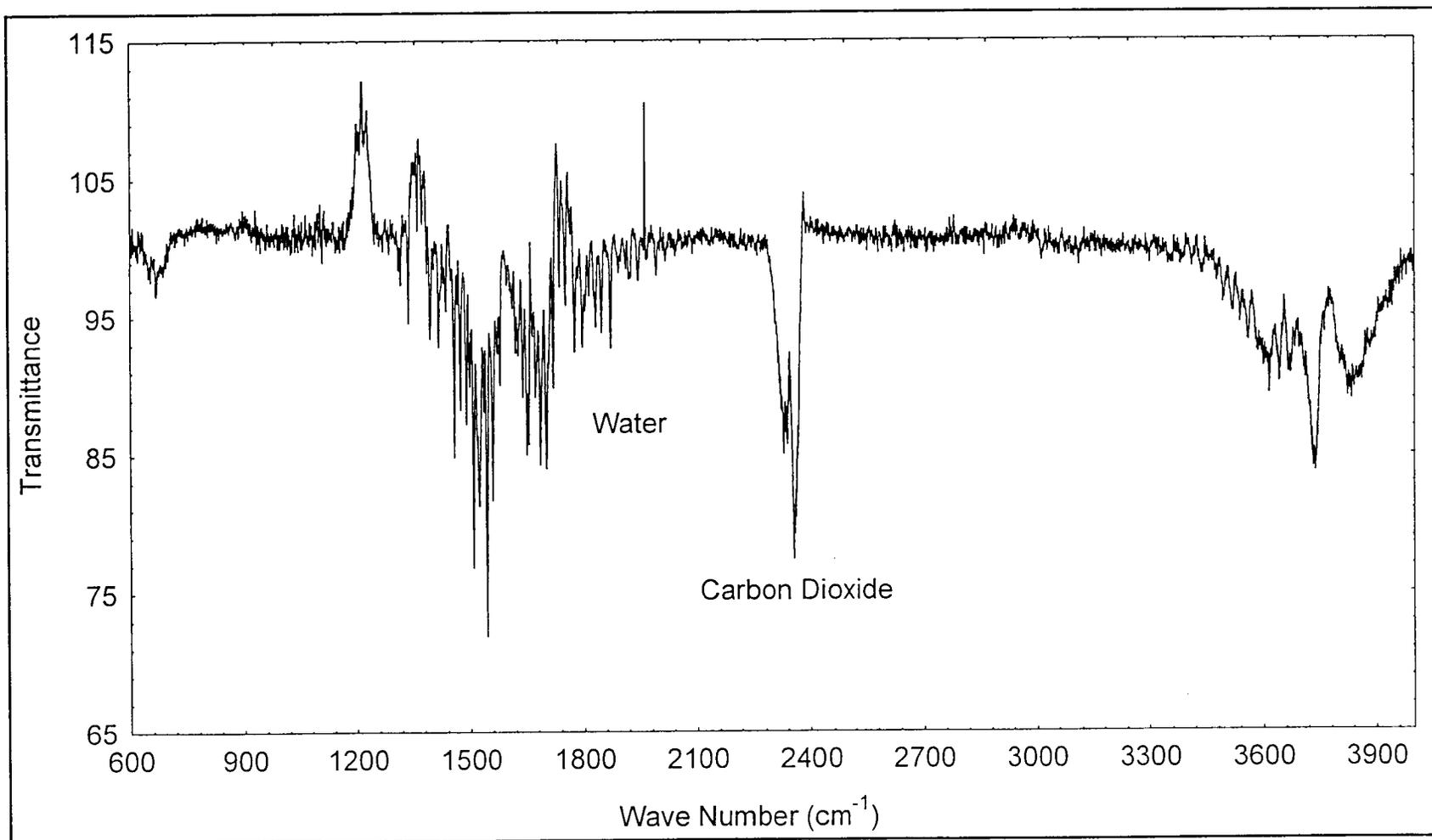
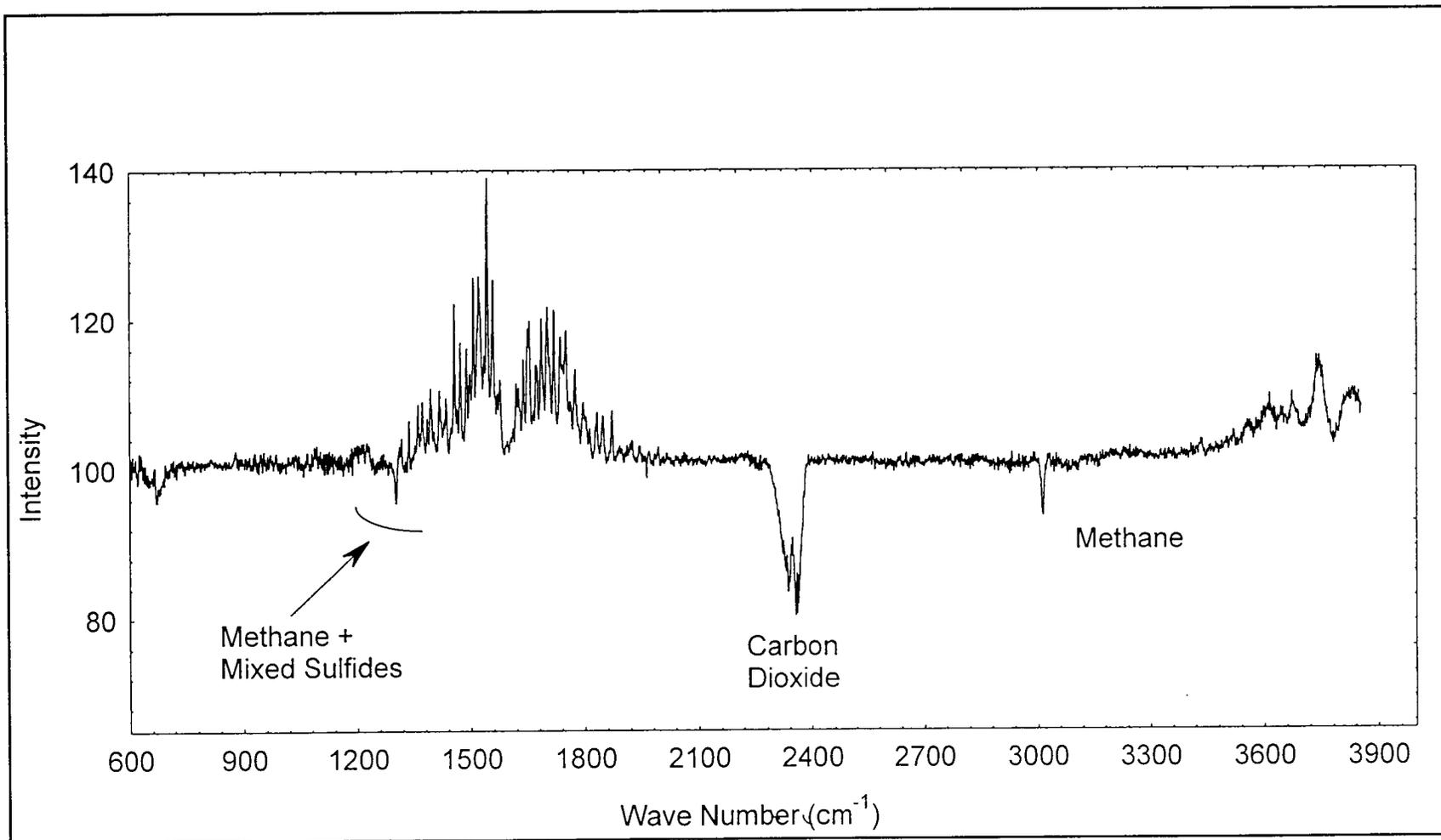Figure 7. Headspace Gases from the Drained/Oxidized Microcosm by FTIR

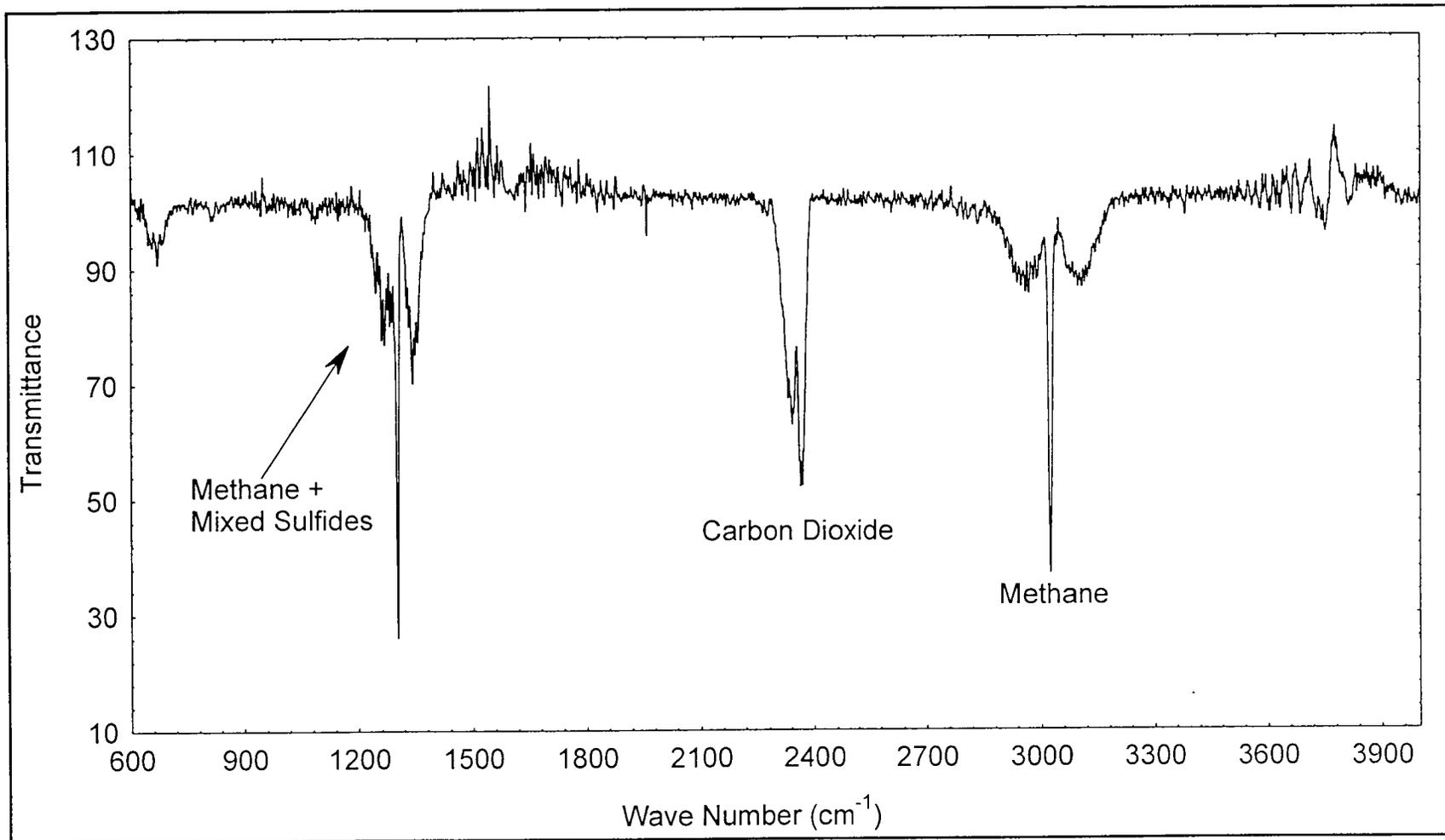Figure 8. Headspace Gases from the Tidal Microcosm by FTIR

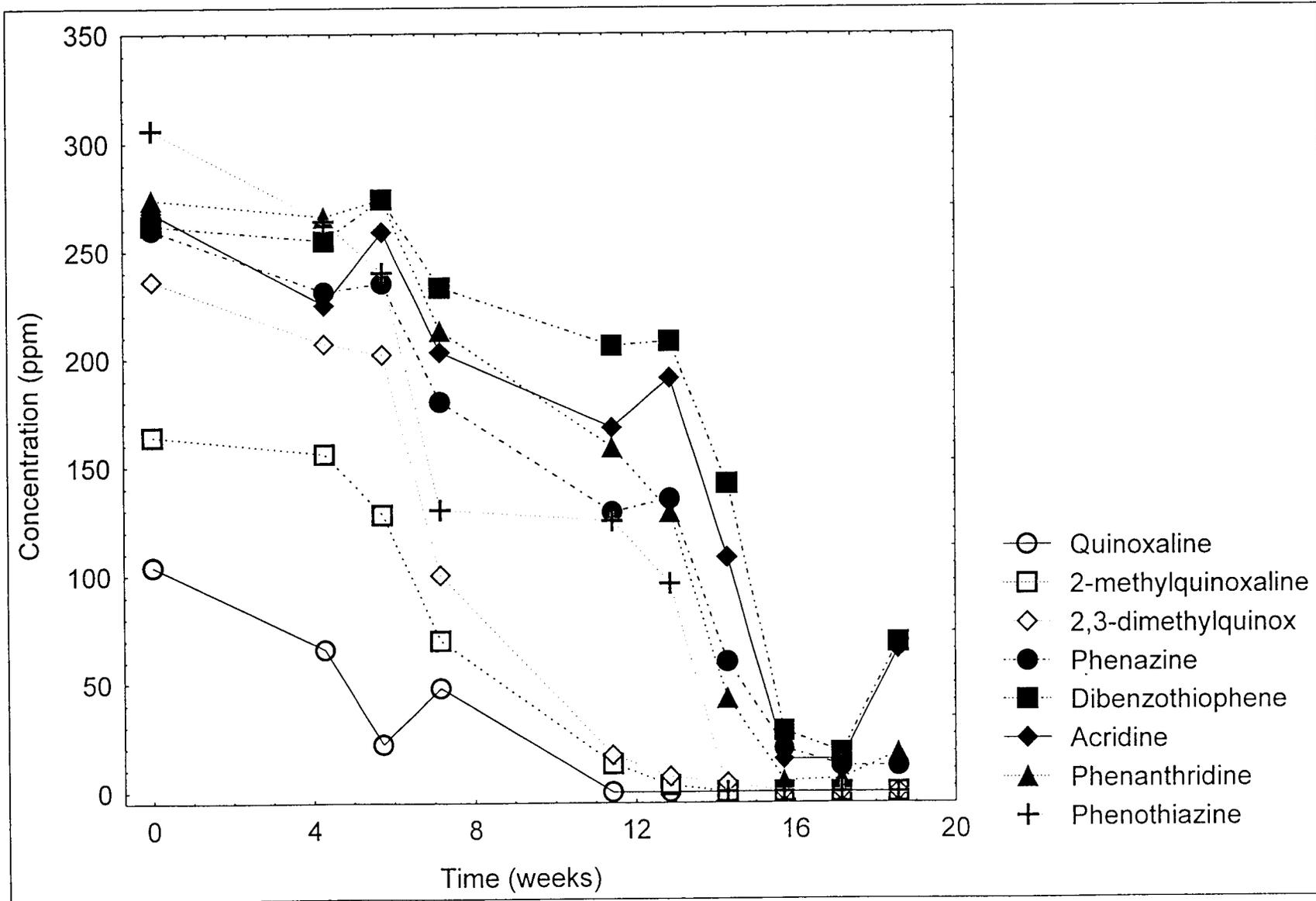Figure 9. Headspace Gases in the Flooded/Reduced Microcosm by FTIR

Figure 10. NOSH Transformation Profiles from the Drained/Oxidized Micrososm
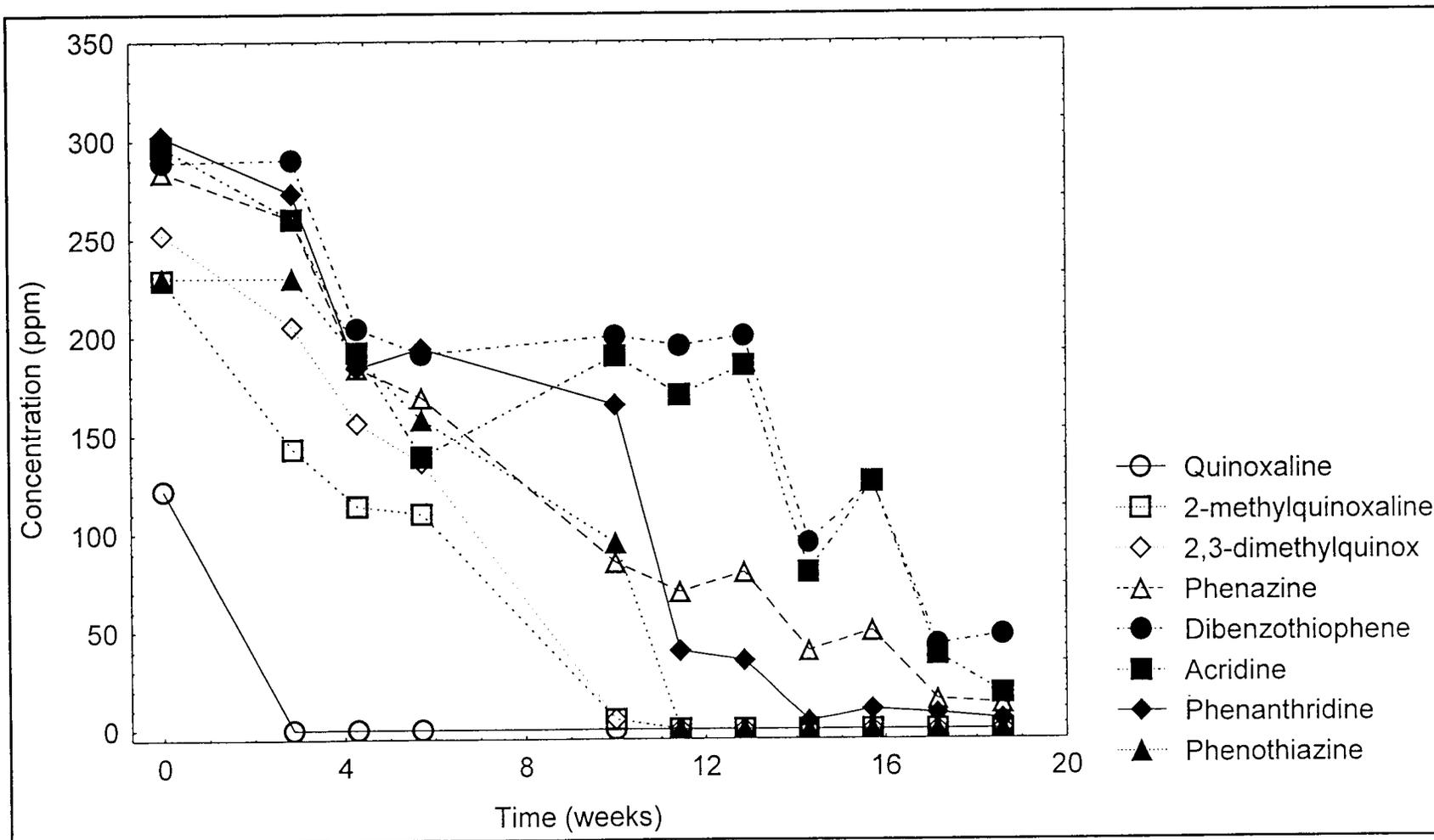
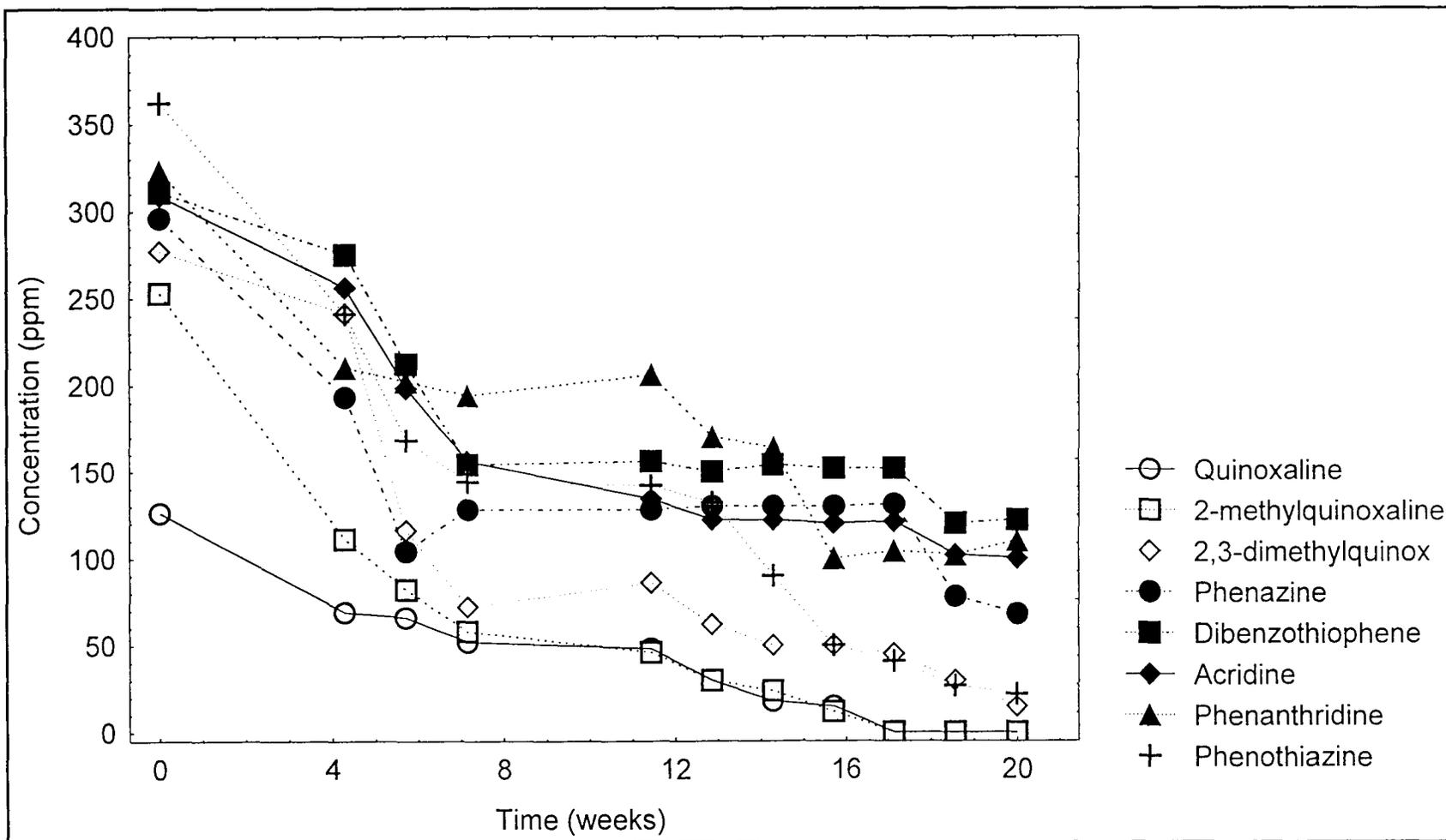Figure 11. NOSH Transformation Profiles for the Tidal Microcosm

Figure 12. NOSH Transformation Profiles for the Flooded/Reduced Microcosm

accommodate prevailing hydroperiods, including tides and seasonal events. For most settings where NOSH and AH contamination is a problem, the well-drained/oxidized approach is not an option unless complete excavation and reclamation of the system is envisaged.

In an abstract sense, the use of hydroperiodicity for simultaneous chemical remediation and ecological recovery of polluted systems seems promising in light of this and subsequent work (Catallo 1999). It would involve, at the very least, attempts to optimize the tidal volume of the wetland without compromising its integrity. Obviously, this kind of undertaking in a real wetland would involve an integrated set of engineering interventions that encompass hydrologic, sedimentologic, and plant-system variables in a progressive sense. The same is true of the use of marginally-contaminated dredge materials for coastal habitat restoration projects. The author is aware of no actual cases in which this has been successfully attempted or contemplated. Much further ecological study is called for in mesocosms and other controlled settings where variables and causal relationships can be identified and ranked with respect to holistic endpoints including, but not limited to, pollutant transformation.

Citations

Asher, H. B. 1983. Causal Modeling. SAGE University Paper 3, Sage Publishers, Newbury Park, CA. ISBN 0-8039-0654-4.

Brockwell, P. J. and R. J. Davis. 1991. Time Series: Theory and Methods. Springer Verlag, NY.

Beyers, R. J. and H.T. Odum. 1993. Ecological Microcosms. Springer Advanced Texts in Life Sciences, (D. E. Reichle, Ed.) Springer-Verlag NY, 557 pp.

Catallo, W. J. 1999. Hourly and daily variation of sediment redox potential in tidal wetland sediments. USGS Biological Resources Division, Biological Science Report, USGS/BRD/BSR-01-1999.

Catallo, W. J., R.P. Gambrell, K. R. Reddy, J. H. Pardue, J. Blankemeyer, and B Pugesek. 1999. Biogeochemical Processes, In: *Ecotoxicology and Risk Assessment for Wetlands*, ISBN: 1-880611-16-3; SETAC Special Publication Series, SETAC Foundation for Environmental Education, Pensacola FL, pp. 27-69.

Catallo, W. J and T. Junk. 1998. United States Patent # 5,830,763 (11/3/98). Process for Preparing Deuterium Tagged Compounds.

Catallo, W. J. 1996a. Transformation of N-, O-, and S- heterocycles (NOSHs) in estuarine sediments: Effects of redox potential and particle size." *Chemosphere:* 33/12:2543-2563.

Catallo, W. J. 1996b. Bioremediation: statistical and analytical needs, 1996b. In: C.E. Proffitt and P. F. Rosigno (eds.). Proceedings - Gulf of Mexico and Caribbean Oil Spills in Coastal Ecostystems: Assessing Effects, Natural Recovery, and Progress in Remediation Research. OCS Study/MMS 95-0063. U.S. Department of Interior, Mineral Management Service, Gulf of Mexico Region, New Orleans LA, pp. 109-129.

Catallo, W. J., W. Henk, L. Younger, O. Mills, D. J. Thiele, and S. P. Meyers. 1996c. Trace metal uptake by *Pichia spartinae*, an endosymbiotic yeast in the salt marsh cord grass, *Spartina alterniflora. Chemistry and Ecology,* 13(2):113-131.

Catallo, W. J., D. H. Hoover, and D. Vargas. 1994. Effects of quinoline on ultrastructure in *Paramecium caudatum* and the protective effects of dissolved calcium, *Aquatic Toxicol.,* 29(324):291-303.

Catallo, W. J. and R. P. Gambrell. 1994. Fates and Effects of N-, O-, and S- Heterocycles (NOSHs) from Petroleum and Pyrogenic Sources in Marine Sediments. Final Grant Completion Report, OCS Study/MMS 674-2010. U.S. Dept. of the Interior, Minerals Management Service, Gulf of Mexico OCS Regional Office, New Orleans, La. 72 pp.

Chikashita, H. , S. Komazawa, and N. J. Ishimoto. 1989. Bull. Soc. Jpn , 1989, 62, 1215.

Junk T. and W. J. Catallo. 1996. Preparative supercritical deuterium exchange in arenes and heterocycles. *Tetrahedron Letts.* 37:3445.

Junk, T. and W. J. Catallo. 1997. Organic reactions in supercritical polar fluids. *Chemical Soc. Rev.*, 26:401-406. Royal Society of Chemists (U.K.).

Mallat, S. 1998. A Wavelet Tour of Signal Processing. Academic Press, NY 577 pp. ISBN 0-12-466605-1.

Odum, H. T. 1983 Ecological and General Systems. University of Colorado Press, Boulder CO, 644 pp.

Turov, Y. P. , N. N. Gerasimova, T. A. Sagschenko, and O.A. Bieko. 1987. Group composition of the low molecular weight nitrogen bases of Samotlor crude oil. Petr. Chem. USSR. 27(1):20-25.

Warshawsky, D. 1992. Environmental sources, carcinogenicity, mutagenicity, metabolism, and DNA binding of nitrogen and sulfur heterocyclic aromatics. Environ. Carcino. Ecotox. Revs. C(10):1-71.

The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.