Environ. Sci. Technol. **2006**, *40,* 1916−1924

Compound-Specific Isotope Analysis Coupled with Multivariate Statistics to Source-Apportion Hydrocarbon Mixtures

THOMAS J. BOYD,*,†
CHRISTOPHER L. OSBURN,†
KEVIN J. JOHNSON,‡
KERI B. BIRGL,†,§ AND
RICHARD B. COFFIN†

Marine Biogeochemistry Section and Chemical Sensing Section, U.S. Naval Research Laboratory, Washington, DC, 20375

Compound Specific Isotope Analysis (CSIA) has been shown to be a useful tool for assessing biodegradation, volatilization, and hydrocarbon degradation. One major advantage of this technique is that it does not rely on determining absolute or relative abundances of individual components of a hydrocarbon mixture which may change considerably during weathering processes. However, attempts to use isotopic values for linking sources to spilled or otherwise unknown hydrocarbons have been hampered by the lack of a robust and rigorous statistical method for testing the hypothesis that two samples are or are not the same. Univariate tests are prone to Type I and Type II error, and current means of correcting error make hypothesis testing of CSIA source-apportionment data problematic. Multivariate statistical tests are more appropriate for use in CSIA data. However, many multivariate statistical tests require high numbers of replicate measurements. Due to the high precision of IRMS instruments and the high cost of CSIA analysis, it is impractical, and often unnecessary, to perform many replicate analyses. In this paper, a method is presented whereby triplicate CSIA information can be projected in a simplified data-space, enabling multivariate analysis of variance (MANOVA) and highly precise testing of hypotheses between unknowns and putative sources. The method relies on performing pairwise principal components analysis (PCA), then performing a MANOVA upon the principal component variables (for instance, three, using triplicate analyses) which capture most of the variability in the original data set. A probability value is obtained allowing the investigator to state whether there is a statistical difference between two individual samples. A protocol is also presented whereby results of the coupled pairwise PCA−MANOVA analysis are used to down-select putative sources for other analysis of variance methods (i.e., PCA on a subset of the original data) and hierarchical clustering to look for relationships among samples which are not significantly different. A Monte Carlo simulation of a 10 variable data set; tanks used to store, distribute, and offload fuels from Navy vessels; and a series of spilled oil samples and local tug boats from Norfolk, VA (U.S.A.) were subjected to CSIA and the statistical analyses described in this manuscript, and the results are presented. The analysis techniques described herein combined with traditional forensic analyses provide a collection of tools suitable for source-apportionment of hydrocarbons and any organic compound amenable to GC-combustion-IRMS.

Introduction

Release of hydrocarbon contaminants into the environment is a significant problem (cf., http://www.nrc.uscg.mil/incident97-02.html), not only from an environmental hazard and cleanup standpoint, but also in terms liability assessment. Releases often occur from multiple sources and, in the case of on-water spills, are subject to complex transport and sedimentation processes. From a forensics and legal perspective, there is a considerable need to distinguish spill or release hydrocarbons from generalized nonpoint source contamination. In practice, the assignment of liability for released hydrocarbons usually involves collecting multiple lines of evidence, then using different chemical analyses to test a given set of scenarios (e.g., ref *1*). Advances in instrumentation have allowed the collection of these multiple types of data, generally at ever-increasing resolution. However, an increase in the amount of available data has not always proved a boon because rigorous and valid interpretation becomes increasingly more complicated. Investigators have turned to the application of multivariate statistics and chemometric approaches to help understand complex chemical data (cf., ref *2*).

To source-apportion hydrocarbons, a number of methods have been reported over the past 10 years. Generalized approaches pinpointing individual constituents of spilled hydrocarbons using gas chromatography (GC) and GC−mass spectrometry (GCMS) have been employed by the US Coast Guard and other agencies in the U.S. (*3*, *4*). Refinements in both chemical techniques, such as comprehensive two-dimension gas chromatography (*5*), and data collection and processing, such as GCMS with multiple single ion monitoring (*6*) or biomarker analysis (*1*, *7*) have provided analytical methodologies for apportioning hydrocarbons to anthropogenic or natural sources. Real advances have been made recently in applying multivariate statistics to chemical data (*1*, *8*) culminating with recent work (*9*) in which statistical probabilities can be assigned to matches between spilled oils and potential sources, providing a defensible framework with which to assess liability.

One biogeochemical property of hydrocarbons that has had less chemometric attention is the application of stable carbon isotopes to environmental forensics. Stable isotope analyses have been developed to study sources and cycling of inorganic and organic molecular compounds in natural ecosystems (*10*−*12*). The successes in applying stable isotope analysis to understand carbon cycling in natural environments offers hope that intrinsic variation in hydrocarbon isotopic compositions might provide another means of apportioning sources. Due to advances in instrumentation coupling separation methods (i.e., GC) with isotope ratio measurements, compound-specific isotope analysis (CSIA) has been applied to understand a number of biogeochemical cycles. Using this technique, individual components in a

---

* Corresponding Author: Phone: 202-404-6424. Fax: 202-404-8515. E-mail: thomas.boyd@nrl.navy.mil.

† Marine Biogeochemistry Section.

‡ Chemical Sensing Section.

§ Present address: Department of Health and Hospitals, Office of Public Health, Laboratory Services, 325 Loyola Avenue, Room 709, New Orleans, LA 70112.

10.1021/es050975p

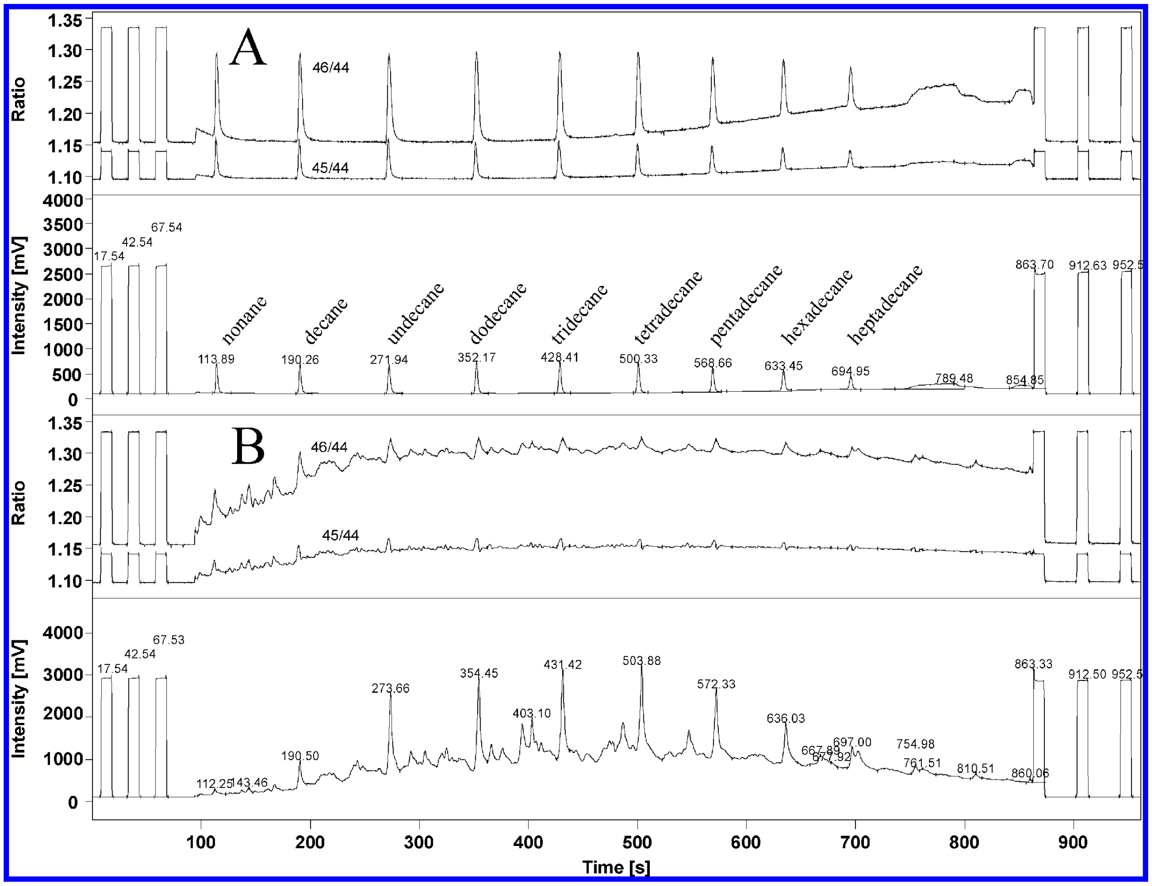

FIGURE 1. Sample chromatograms for *n*-Alkanes C_9 to C_{17} $\delta^{13}C$ analysis. A. Authenticated standards, B. Field sample run, *Mobil Facility* fuel (see text for explanation).

mixture are separated, combusted on-line and transferred to an isotope ratio mass spectrometer (IRMS), in some cases even with conformational data supplied by split flow to an ion trap mass spectrometer (ITMS). With CSIA, the isotope ratio of each component within a mixture can be measured and it can be accurately inferred in a concentration-independent fashion, if two hydrocarbon samples are from different sources.

The isotope ratios of individual components of a hydrocarbon mixture are set during plant photosynthesis (forming the organic matter), subsequent petrogenesis and refinery processes (*13*). As a result, a discreet source of oil will have a unique compound-specific isotope signature (*14*, *15*). Therefore, even though concentrations of individual components (or ratios of components) within the oil or fuel mixture may change over time—due to differential dilution, volatilization, or biodegradation—the isotope ratios of the individual components may remain the same. This may not be the case for some types of hydrocarbons, particularly volatiles (for instance benzene, toluene, ethylbenzene, and xylenes) and chlorinated hydrocarbons (i.e., chloroethylenes). Much of the CSIA literature revolves around using biodegradative fractionation factors for these constituents to confirm on-site bioremediation (*13*, *16*). In most aerobic environments, nonvolatile and semi-volatile hydrocarbons (aliphatic and polynuclear aromatics) appear to have little isotopic fractionation during biodegradation, vaporization, or photolytic conversion (*17*–*19*). This means that a discreet source of hydrocarbon (i.e., a singular spill) should provide a defined CSI signature that remains with the spill hydrocarbons irrespective of changes in concentration or concentration ratios, in any location the spill migrates.

The use of CSIA has just begun to be applied for source apportionment. In several studies, different sources of hydrocarbons were determined by comparing ratios of individual components of the hydrocarbon mixture (*20*, *21*). This approach has been fruitful as subtle variations in natural carbon isotope ratios allow for direct comparison of individual components. In addition to individual isotope comparisons, two-dimension comparisons are now more common coupling both $\delta^{13}C$ and $\delta^{15}N$ or δD analysis (*22*, *23*). What has been lacking is a comprehensive chemometric approach to isotope analysis providing a robust statistical framework for source-apportioning hydrocarbon mixtures.

In this manuscript, we report on a method for analyzing CSI values by a group of multivariate statistical tests which provides statistical probability that any two samples (or common mixtures of hydrocarbons) are the same; through principal components analysis (PCA) on the entire sample set and a reduced set based on match probabilities, a graphical representation of possible matches between unknowns and putative sources; and, finally, using hierarchical clustering, a potential relation between unknowns and potential source hydrocarbons.

Methodology

Standard *n*-Alkane Source. *n*-Alkane standards analyzed on the GC/ITMS/IRMS were purchased individually from Sigma-Aldrich, mixed in equal volume proportions, and diluted at a 1:50 ratio in methylene chloride. Chromatographic resolution was sufficient for resolving nonane through heptadecane (Figure 1A). Standard samples were run at least once with each batch of experimental samples (usually at the beginning and end of each batch). The average value for each standard (11 replicates) was used to calibrate values for each experimental batch.

Monte Carlo Simulation. To understand the operating parameters of the statistical method, a series of simulated

TABLE 1. Results of MANOVA Using the First Three Principal Component Scores (PCS) with All Ten Replicates and PCA−MANOVA Using Random-selected Triplicates from Simulated 10 Component, 10 Replicate δ^{13}C Data

variation	difference	P	*P* (1st 3 PCS)	PCA-Manova *P*(±capture)
2‰ variation	no difference	0.79	0.49	0.28 ± 0.036
2‰ variation	each component different by ≥ 2 ‰	$<10^{-5}$	$<10^{-5}$	0.003 ± 0.00001
2‰ variation	2 varied by 0.5 ‰	0.78	0.25	0.76 ± 0.003
2‰ variation	2 varied by 2 ‰	0.022	0.00070	0.064 ± 0.0008
2‰ variation	4 varied by 1 ‰	0.0077	0.00027	0.099 ± 0.004
1‰ variation	No difference	0.23	0.49	0.48 ± 0.06
1‰ variation	2 varied by 0.5 ‰	$<10^{-4}$	$<10^{-5}$	0.015 ± 0.0004
1‰ variation	4 varied by 1 ‰	$<10^{-5}$	$<10^{-5}$	0.0071 ± 0.0005
1‰ variation	2 varied by 1 ‰, 2 by 2 ‰	$<10^{-5}$	$<10^{-5}$	$0.0001 \pm <10^{-5}$

[a] Variation denotes the variation specified for the random number generator (either 1 or 2‰). Difference indicates the manipulation between two simulated 10 replicate, 10 component data sets.

δ^{13}C data sets were created for comparison. These data sets consisted of 10 hydrocarbon components. The RAND function of Microsoft Excel was used to generate uniformly random δ^{13}C values with either a 1 or 2‰ range for each component. The overall range of values between variables was from −18 to −31‰. After generating a data set with 10 replicates of the same hypothetical sample, a second set was generated that simulated replicates of different samples by varying two through four components by a known offset, or in some instances generating a second random set with the same parameters (Table 1 and supplemental data). These two 10 × 10 matrixes were then exported to Matlab and analyzed by standard multivariate analysis of variance (MANOVA). The *P* value was recorded. Next, the two 10 × 10 matrixes were reduced by PCA analysis. The first three columns of PCA scores were then analyzed by MANOVA (to determine the applicability of the PCA−MANOVA described below with no restriction on replicates). The *P* value was recorded. Finally, the PCA−MANOVA method described below was applied to three replicates from each simulated 10 × 10 data set (giving a 3 × 10 test). The generated *P* value was also recorded for comparison (see Results and Discussion and Table 1). The simulated data is included as Supporting Information.

Fuel Tank Sampling. To determine if source differences could be detected, diesel fuel tanks at the Norfolk Naval Ship Yard (Craney Island) in Norfolk, VA (U.S.A.) were sampled on 21 February and 17 April 2001. Sampling was conducted through the top tank port with 100-ml sample containers attached to a lowering line. These tanks may hold “fresh” fuel. However, tanks are often back-fed from ships as well as inter-mixed, depending on local needs. Fuel samples were diluted 1:50 with methylene chloride before injection in the GC/ITMS−IRMS instrument.

Fuel Spill Samples. The Marine Environmental Response Branch, Marine Safety Office Hampton Roads, USCG provided samples consisting of neat floating hydrocarbons from a diesel spill discovered on the water column surface near the Berkley Bridge on the East Branch of the Elizabeth River in Norfolk, VA (36.84N × −76.28W). The spill (USCG case number 01000232) was discovered on 04 January 2001 and consisted of approximately 1500 gallons of diesel fuel. Three distinct spill samples were collected on the north side of the Elizabeth River (waterside), south side (Metro Machine) and directly under the Berkeley Bridge (Berkeley Bridge) which spans the river. Shipboard samples were collected by directly sampling tanks and bilges of the following local tugs and fuel depots: *Mobil Facility*, *Tarmac I*, *Tidewater Star*, *Bay King*, *Bay Eagle*, *Bay Hawk* (day tanks), *Bay Hawk* (port and starboard tanks), *Baytide Storage*, *Baytide* (day tanks), *Bay Hawk*, *Portsmouth Star*, *Arapano*, *Seletar Hope*, *Alert* (bilge), *Alert* (day tanks), and the *Sea Star*. Samples were sent directly to the Coast Guard analytical lab. Splits from these samples were then sent to NRL for GC−IRMS (CSIA) analysis. A representative chromatograph is shown (Figure 1B).

Chromatography. Methylene chloride-diluted (1:50) neat *n*-alkanes were injected (2 μL) into a Varian model 3400 gas chromatograph (GC) for separation on a 60 m, 250 μm diameter Supleco SPB-05 capillary column with 250 μm film thickness. The injector was operated in splitless mode in order to minimize potential isotope fractionation in the inlet (*24*). Column operating conditions were as follows: 40 °C for 1.00 min, ramp to 220 °C at 11.2 °C min^{-1} for 16.07 min, ramp to 290 °C at 35 °C min^{-1} for 2.00 min. The gas stream from the column was split with 80% of the flow directed through a Finnigan MAT combustion interface to a Finnigan MAT Delta S isotope ratio mass spectrometer (IRMS). The remaining flow was directed to a Finnigan MAT Magnum Ion Trap MS (ITMS) for conformational analysis. Known reference gas samples ($\delta^{13}C_{VPDB} = -45.01$) were injected into the source area of the IRMS in triplicate before and after sample introduction. Retention times were calculated based on known *n*-alkane standards. Field *n*-alkane samples were compared to the standard runs and verified using the National Institute of Standards and Technology (NIST) library using the ITMS and AMDIS (www.nist.gov) software. δ^{13}C values were calculated as

$$\delta^{13}C = \left[\frac{R_s}{R_{std}} - 1\right]1000(‰) \tag{1}$$

where δ^{13}C is the stable carbon isotope ratio, *R* is the $^{13}C/^{12}C$ for stable carbon, s is the sample and std is the standard. Samples were referenced against CO_2 calibrated to Vienna PeeDee Belmenite (VPBD). All peak voltages were well within instrument linearity (1−6 V), or were discarded in subsequent analysis.

Statistical Analysis. Data from triplicate analyses were transferred to Matlab in which multivariate statistical tests were conducted using the statistical toolbox and custom-written function(s) described below. An initial data reduction was performed whereby individual hydrocarbon components not detected in all samples to be tested were removed from the set. This often led to the removal of nonane (C_9) and decane (C_{10}) from analysis. Data from CSIA lend themselves perfectly to multivariate analyses as each component of the mixture represents an independent variable impacted by initial enzymatic selection and diagenetic processing. As far as rejecting individual samples as a match for an unknown, the most powerful statistical test is an analysis of variance (ANOVA). With multivariate data, there is the option of performing one ANOVA test per variable and then pooling the results, or the option of performing a MANOVA. For this application, MANOVA is preferred, as error could be introduced by pooling the results of multiple variable-wise ANOVA tests. Specifically, while a Bonferroni correction can be applied to lower the significance level of each individual test to appropriately reduce the overall Type I error rate associated with pooling multiple ANOVA tests, this comes at the expense of increasing Type II errors (*25*). Unfortunately, there are conflicting forces at work in adapting data taken from typical CSIA to MANOVA analyses. The robustness of MANOVA is increased by having more variables (i.e., more isotope values individual components of the mixture), however, MANOVA requires that there be at least as many replicates as there are variables and is generally applied to data sets with large numbers of observations. The goal of this work was to provide a robust and workable solution for statistical postprocessing of CSIA data. It is impractical and expensive to perform 6−10 or more replicates using GC−IRMS instrumentation. Therefore, a method was needed to "adapt" data from CSIA to a form suitable for hypothesis testing. A number of attempts to model data variability from multiple injections of known standards proved unsatisfactory under varying conditions (i.e., closely versus nonclosely related sources).

This difficulty seems inherent to disciplines outside large population studies and has recently been addressed by Läuter et al. (*26*, *27*). Further adaptation of statistical methods for data with a small number of observations but a large number of variables was recently presented by Langsrud (*28*). These papers have demonstrated that one can re-project original data into decomposed variables and thus replace original data with new data capturing variability of the original data set, without affecting the results of subsequent multivariate tests, such as MANOVA (*26*). Because we assume that isotopic variation among components of a source hydrocarbon is not the result of any single factor, rather a combination of natural variation and refinery processing, a principal components analysis (PCA) is appropriate for describing the component variability and projecting that variability into a new data-space.

Although there is less need to normalize or otherwise scale δ^{13}C values (commonly done to reduce bias in PCA) as they are generally all the same order of magnitude, techniques such as mean-centering ensure direct comparison of the relative difference between samples rather than the distances of different samples from the origin of the measurement space. The function of PCA is to project data onto a lower-dimensional subspace composed of orthogonal axes that most efficiently describe the variation within the data. These axes are ordered according to how much of the variance of the original data they contain (*29*). Thus, we can initially assume that most of the data differences between multiple components in two mixtures will be captured in the first several principal components (PCs). If triplicate analyses are factored by PCA in a sample-wise fashion, i.e., triplicate vs triplicate, any significant sample to sample variability in the original data set will be represented in the first few principal components. This is because there is theoretically only one significant source of variation in such a data set, provided, of course, that the variance associated with each measurement in the original data is roughly the same.

In sample-wise comparisons we found an average of 98% ($n = 172$) of initial sample variability captured in the first three PCs. Therefore, our processing scheme involves first performing sample-wise PCA, then performing a MANOVA on the first three PCs (i.e., the number of original replicates) of the factored data set to test sample difference. Langsrud (*28*), suggests that variability captured in PCs not used in subsequent hypothesis testing should be presented along with probability values. In this work, we have propagated error in capturing only ~98% of the original sample variation by using the uncaptured variability (in PCs >3 for triplicate analysis) to create confidence intervals around probability values. For example, if 98% of the original variability was captured by sample-wise PCA and the subsequent MANOVA produced a probability of 0.10, a 2% confidence interval was calculated (i.e., ±0.002).

A Matlab program was created to cycle through all data and perform individual sample-wise PCA−MANOVA comparisons in the above manner. The number of individual tests (n) can be calculated as the sum of all integers less than the number of samples:

$$n = \sum_{Samples-1}^{j=1} j \qquad (2)$$

The Matlab program calls a custom-written function producing a new data table specifying the test (i.e., sample 1 vs sample 2, and so on), the probability value (P), the capture percentage (CP) of the original variability, and the confidence interval. These data can then be copied to Microsoft Excel so that values can be sorted based on samples, P values, and so forth. The original data from sample tests having P values indicative of no statistical difference between samples ($P <$ 0.05) were then collated and analyzed by standard Matlab PCA and hierarchical clustering analyses (in the statistics toolbox). Samples having the largest P values (i.e., the greatest probability that the mean difference is not due to chance), the closest proximity to one another in PCA plots, and close hierarchical clustering were considered the greatest possible candidate matches.

The PCA−MANOVA process tests the probability of any two samples being statistically the same. In the PCA alone test, in which all samples having a P value greater than 0.05 in a given data set were included (not multiple pairwise analysis), variability in the original data set is used to screen for relation. The final multivariate test involved hierarchical clustering. This technique attempts to cluster relation among samples having multivariate data. Again, data from sample combinations having a P value greater than 0.05 were considered candidate matches. These data were averaged for each variable of each sample, then the means were processed using Matlab's pdist (p distribution) and linkage functions in the statistics toolbox. Output from this analysis was plotted in a dendrogram and putative matches (passing MANOVA and PCA critera) were verified by falling into hierarchical clusters. Clustering was used as an additional line of evidence apportioning unknown samples to a particular known source. The overall statistical processing routine (Figure 1) loosely follows the decision-making flowchart presented by Christensen et al., (*9*) whereby subsequent tests refine matches in order to arrive at the best possible statistically defensible apportionment.

Results and Discussion

Standards. The individual δ^{13}C for standard mix of *n*-alkanes consisting of decane (C_{10}), undecane (C_{11}), dodecane (C_{12}), tridecane (C_{13}), tetradecane (C_{14}), pentadecane (C_{15}), hexadecane (C_{16}), and heptadecane (C_{17}) ranged from −32.4 to −24.1‰ through 11 runs and resulted in a standard error (s.e.) from ±0.10 to ±0.14. The largest standard errors were observed in C_{12} and C_{13} where the peaks were most difficult to resolve. The average for the standard error of all eight *n*-alkanes was ±0.13‰. Previously reported values for BTEX, TNT, and PAHs have been measured in the range of ±0.3, ±0.1, and ±0.1‰, respectively (*21*, *22*, *30*). During the course of the running experimental batches (for all samples reported), the deviations from the standard averages ranged as high as 0.7‰.

TABLE II.[a] Results of PCA−MANOVA Significance Tests for Sequential Sampling of Norfolk Fuel Storage Tanks[a]

sample 1	sample 2	P	capture %	±
tank1 t1	tank1 t2	0.055	92.0	0.0044
tank2 t1	tank2 t2	0.343	92.5	0.026
tank3 t1	tank3 t2	0.029	94.1	0.0017
tank4 t1	tank4 t2	0.938	86.0	0.13
tank 1 t1	tank 2 t1	0.422	97.1	0.012
tank 1 t1	tank 3 t1	0.052	91.6	0.0044
tank 1 t1	tank 4 t1	0.857	89.9	0.087
tank 2 t1	tank 3 t1	0.328	96.4	0.012
tank 2 t1	tank 4 t1	0.933	84.2	0.15
tank 3 t1	tank 4 t1	0.683	92.5	0.051

[a] Results reflect C10 − C17 hydrocarbons.

Monte Carlo Simulation. By simulating δ^{13}C data with known levels of precision (i.e., 1 or 2‰), it was possible to determine under what parameters the described PCA−MANOVA tests are able to provide reliable estimates of the "true" *P* value. Using only three of the 10 replicates, at both 1 and 2‰ range for each component, PCA−MANOVA correctly showed no statistical difference in simulated replicate δ^{13}C values of the same sample (Table 1). At a 2‰ range for each component with 2 components varied by 0.5‰ between data sets, none of the statistical treatments showed a significant difference between the data sets. At 2‰ range per component and 2 components varied by 2‰ or 4 components varied by 1‰, both a MANOVA on the 10 × 10 data sets and a MANOVA on the first three principal components of the data sets (10 × 3 comparison) showed a statistical difference ($P < 0.05$). However, when three of the 10 replicates were chosen for the PCA−MANOVA method described in the manuscript, there was no statistical difference between the data sets ($P > 0.05$) (Table 1).

When the simulated data sets had a 1‰ range of precision for each component, the PCA−MANOVA method described here performed adequately for all tested scenarios. The PCA−MANOVA method was able to show a significant difference between triplicate samples taken from the 10 replicate data set with as small as a 0.5‰ shift in only two of the 10 components (Table 1). We conclude from this simulation that analytical precision should be $\leq$1‰ to adequately use the PCA−MANOVA method for determining statistical differences between multivariate CSIA data. Typical values for precision are less than 1‰, so this method should have broad applicability (cf., ref *31*). It should also be noted that the statistical processing routine presented here does not absolutely require standardization to known isotopic standards, now routine for accurate δ^{13}C reporting. Because normalization and PCA eventually create centered "new" variables, as long as the processes is systematic, one could reference analytical data to any standard (i.e., arbitrarily set the calibration standard to 0‰ and reference all experimental peaks to it).

Norfolk Tank Samples. Four fuel tanks (*1*−*4*) were sampled on 21 February 2001 and again on 17 April 2001. In addition, fuels from other tanks were sampled to determine the variability expected in Navy fuels. For security reasons, detailed information regarding the exact disposition of fuels is not presentable. Fuel within any given tank was a mixture of the original filling, additional fillings, and backfilling from ships. As most of these fuels were freshly refined, we were able to resolve $C_{10}-C_{17}$ *n*-alkanes. Initial samples (February 01) showed no significant differences between any of the four tanks in pairwise tests (Table 2). In fact, several pairings (Tank 1 and Tank 4, and Tank 2 and Tank 4) had *P* values greater than 0.5 conceivably indicating identical sources.

Data were available for fuel transfers from Tanks 2−4. During the sampling period, Tank 2 had no refilling operations, and did not offer any significant difference in the sampled sources (Table 2). Fuel was offloaded by one ship to Tank 3 during the inter-sample period. Mixing in of this source likely resulted in a significant difference between the temporal samples (Table 2). Tanks 2 and 4 were the most interesting cases encountered in this study. Tank 2 was initially no different than Tank 4 ($P = 0.933$). It had no recorded refilling operations, yet had a lower *P* value (0.343) at t2. We attribute this unusual observation to the lower precision in the Tank 2 and Tank 4 data relative to the other tanks included in the data set (i.e., the average standard deviations). From the Monte Carlo simulation, we observed that lower precision data gave a higher deviation for the true *P* value (i.e., with many replicates). Over the course of 3 days in March 2001, Tank 4 received the offload of three separate ships (roughly 400 000 Gallons). Although we have been unable to verify the source of fuel on these ships, it is typical for fuel offloading to occur after joint deployments. These ships drew fuel from the facility sampled for this study. Records are not available for which tank fueled which ships (only receipts are tracked). However, if these ships drew from a common source (i.e., Tank 4), the source ratios would remain unchanged. Although a controlled study would be more useful in a forensics application, i.e., tracking both fueling and receipts from one tank over time, the purpose of this study was primarily to determine if statistical differences could be detected from tank to tank and over time.

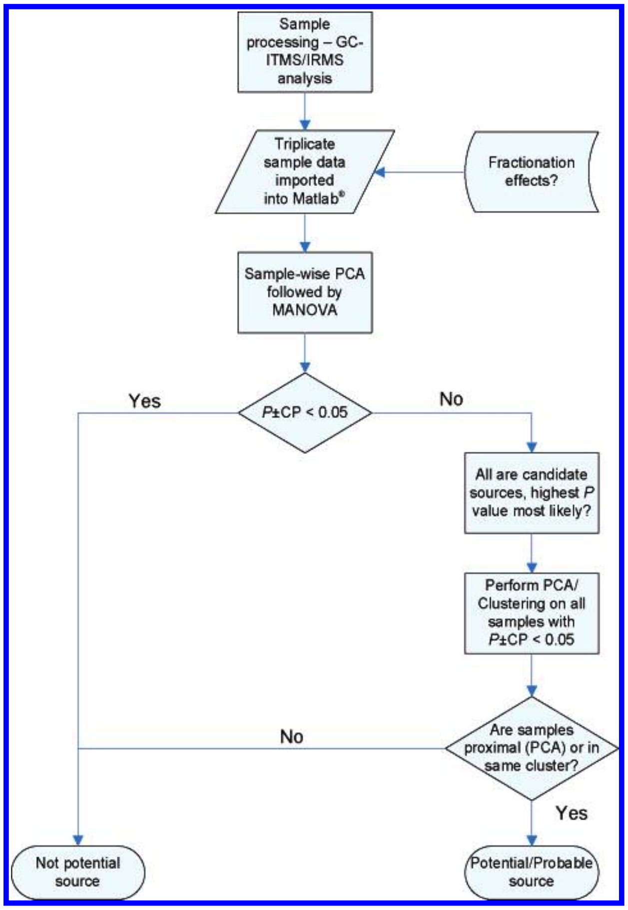

FIGURE 2. Statistical processing flowchart. Fractionation effects are addressed in the discussion section.

Fuel Spill in Elizabeth River. In developing this analysis, the U.S. Coast Guard expressed interest in coupling the capabilities of CSIA along with their current approach to identify potential spill sources after in-water releases. The Coast Guard findings for this spill were inconclusive. Several

TABLE 3. Results of PCA−MANOVA Significance Tests for Norfolk Spill Samples

sample 1	sample 2	P	capture %	±
Arapano	spill Metro Machine	0.114	98.2	0.00204
Bay Eagle	spill Metro Machine	0.178	98.7	0.00233
Bay Hawk D/T	spill Metro Machine	0.200	98.7	0.00262
Bay Hawk P&S tanks	spill Metro Machine	0.221	95.3	0.01039
Bay King	spill Metro Machine	0.929	96.1	0.03645
Mobil Facility	spill Metro Machine	0.130	96.4	0.00472
Seletar Hope	spill Metro Machine	0.134	90.5	0.01278
Arapano	spill at Waterside	0.082	99.1	0.00075
Bay Eagle	spill at Waterside	0.330	99.3	0.00234
Bay Hawk D/T	spill at Waterside	0.330	99.3	0.00219
Bay Hawk P&S tanks	spill at Waterside	0.197	99.0	0.00203
Bay King	spill at Waterside	0.165	99.0	0.00160
Mobil Facility	spill at Waterside	0.280	94.4	0.01569
Seletar Hope	spill at Waterside	0.162	97.5	0.00407
Arapano	sheen at Berkeley Bridge	0.015	99.1	0.00014
Bay Eagle	sheen at Berkeley Bridge	0.076	96.7	0.00251
Bay Hawk D/T	sheen at Berkeley Bridge	0.111	97.0	0.00333
Bay Hawk P&S Tanks	sheen at Berkeley Bridge	0.006	98.5	0.00009
Bay King	sheen at Berkeley Bridge	0.001	98.4	0.00002
Mobil Facility	sheen at Berkeley Bridge	0.022	99.2	0.00017
Seletar Hope	sheen at Berkeley Bridge	0.008	99.2	0.00006

[a] Results reflect those samples having *P* values above 0.05 for at least two of the three spill samples. C11 − C17 hydrocarbons were used for this analysis.

tug fuel samples were discounted by virtue of being a different fuel type (*Alert Bilge* and *Sea Star*) while most other samples were found to be light fuel oils with characteristics similar to the spill samples (*32*, *33*). Our statistical analysis started with performing pairwise PCA−MANOVAs for all samples. This amounted to 172 individual tests. *P* values ranged from 0.00094 to 0.93 while the percent capture of variability ranged from 91 to 100%. The resulting probabilities were screened for possible matches using the *P* values indicative of no significant difference (see Figure 2). By sorting the higher *P* values for each spill source, the original 16 suspect sources were reduced to 7, all having at least two out of three comparisons to the three spill sources with no significant difference (i.e., $P < 0.05$) (Table 3). Interestingly, the *Sheen* at Berkeley Bridge was found to be statistically different to both the spill at waterside and the spill at Metro Machine (which were, in turn, found to be statistically the same). The Coast Guard analysis classified all spill samples as slightly weathered. Although there is little evidence to suggest that hydrocarbon in the range used for this study fractionate isotopically during weathering (*19*, *34*), perhaps if the spills were all derived from a common source, some might have occurred. Another explanation is that the *Sheen* at Berkeley Bridge was the result of a separate release. Even though the isotope values for this sample were significantly different than those for the other spill samples, for purposes of this statistical comparison, the *Sheen* at Berkeley Bridge was treated as a source of the original spill.

The putative matches from the PCA−MANOVA analysis were then included in standard PCA (Figure 3A), and hierarchical clustering analyses to assess relatedness (Figure 3B). PCA analysis yielded 71% of variance captured in the first principal component. Twelve percent of initial sample variance was captured in the second principal component and almost 9% in the third component. The spill at Metro Machine and the spill at waterside were both positively loaded with respect to component one, and the *Sheen* at Berkeley Bridge was negatively loaded. The spill at *Metro Machine* was positively loaded with respect to component two while the other spill sources were negatively loaded. *Arapano*, *Bay Hawk* P&S (port and starboard) tanks, and the *Seletar Hope* showed the closest proximity to the spill at Metro Machine and spill at waterside sources in terms of both components, while *Bay Hawk* D/T (day tanks) was centrally loaded between all spill sources (Figure 3A). Based on the PCA analysis, it appears that the *Arapano*, *Bay Hawk* P&S Tanks, the *Seletar Hope,* and *Bay Hawk* D/T are the closest matches. The variability captured in the first two principal components placed *Mobile Facility*, the *Bay King* ,and the *Bay* Eagle a bit further from the sources. While the *Arapano*, the *Bay Hawk* P&S Tanks, and the *Seletar Hope* showed the greatest degree of similarity in PCA loadings to at least two of the spill sources, the PCA−MANOVA analysis indicated that these tugs were significantly different from the *Sheen* at Berkely Bridge (Table 3). The *Bay Eagle* and *Bay Hawk* D/T were the only samples showing no statistical difference for all three spill sources.

For the clustering analysis, the *Bay King* clustered with both the spill at Metro Machine and the spill at waterside. This cluster was in turn clustered with the *Bay Eagle*, *Bay Hawk* D/T, *Bay Hawk* P&S Tanks, and the *Arapano*. The distance of relatedness between the spill at Metro Machine and the spill at waterside and the tugs closest to the *y*-axis is remarkably similar as the horizontal line connecting the spill samples to the tug clusters is only about 0.1 units on the *y*-axis (Figure 3B). This indicates that while these tugs have various relations with each other, they all have virtually the same relation to the spill at waterside. *Mobile Facility* and the *Setelar Hope* are clustered together and only have about 0.1 unit of distance in relation to between the spill at Metro Machine and the spill at waterside. The *Sheen* at Berkely Bridge is the most distinct and shows very distant relation to both the other spills and the tugs used in this analysis.

The multivariate statistical approaches used for this forensic approach (Figure 2) include the ability to assess statistical differences in the collective means of the variables (PCA−MANOVA), the variability in the data set (PCA), and the statistical relation of samples (hierarchical clustering). Piecing together the results of all three multivariate analyses, one could make a strong case for the *Bay Hawk* D/T or *Bay Eagle* based on the fact that these were the only samples that showed no significant difference to all three of the spill sources. These samples also showed a great deal of related-

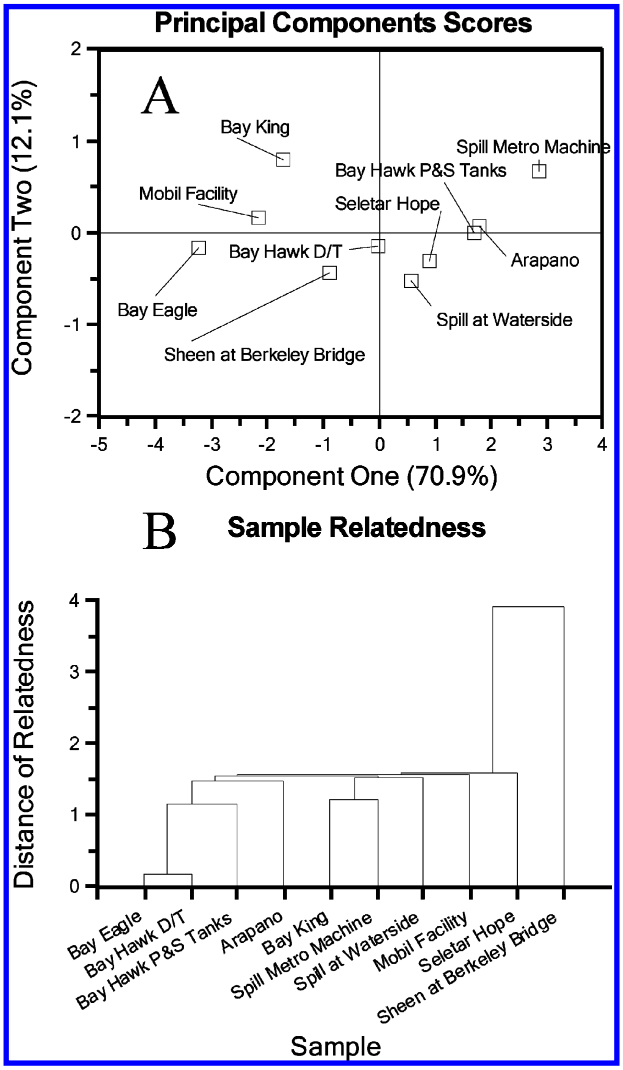

FIGURE 3. PCA (A) and hierarchical cluster (B) analysis of selected Norfolk fuel spill samples.

ness to one another based on the clustering analysis (Figure 3B). The *Bay King* was clustered most closely to the spill at Metro Machine and the spill at waterside (Figure 3B), but was negatively loaded with respect to component one in the PCA analysis, whereas each of these two spill sources was positively loaded (Figure 3B). The *Bay King* also showed the highest P value (0.93) for relation to the spill at Metro Machine, indicating that the samples were virtually the same. However, the *Bay King* was significantly different from the *Sheen* at Berkeley Bridge ($P = 0.001$); if all of the spill sources are considered to be the same, the *Bay King* fails to remain a candidate. If we make the assumption that all three spill samples represent the same source, we would conclude that the best possible match for the leaking tug is either the *Bay Hawk* D/T or the *Bay Eagle*. If we consider weathering a possibility (in terms of changing the isotope ratios), we would include the *Bay King* a strong candidate for the source of the spill.

Fractionation Analysis. Isotopic fractionation due to biodegradation, volatilization and weathering is a concern when attempting to use CSIA to source-apportion hydrocarbons. Many compounds undergo isotopic shifts during these processes and the observation of a predictable fractionation is often used as evidence for biodegradation or other remediation process (see refs *13*, *16*, *35* for recent reviews). In order to use a particular hydrocarbon mixture for source-apportionment, it is critical to understand what processes may introduce fractionation and the degree to which fractionation (if any) occurs. A controlled experiment was conducted by Mansuy et al. (*36*) in which fuel oil hydrocarbons were artificially weathered by evaporation (4 yr), water washing (2 months), and biodegradation (4 months of mixture with sewage sludge). These studies showed that *n*-alkane fractionation due to weathering processes was generally on the order of analytical precision for CSIA and should provide minimal interference in source-apportioning *n*-alkane hydrocarbons using the described method. In another biodegradation assay, no detectable fractionation was observed for *n*-alkanes (*18*). Therefore, we would expect there to be very little fractionation in the samples analyzed for this study. However, if fractionation factor(s) were known for components of a hydrocarbon mixture, measured isotope ratios could be altered mathematically to the pre-fractionated value(s). For instance, if anaerobic benzene biodegradation was known to cause a −3‰ shift in δ^{13}C value in the remaining source pool (cf., ref *35*), one could correct for this and alter the measured source value. Under this scenario, a measured benzene δ^{13}C of −32.0‰ would be corrected to −29.0‰ when apportioning source(s).

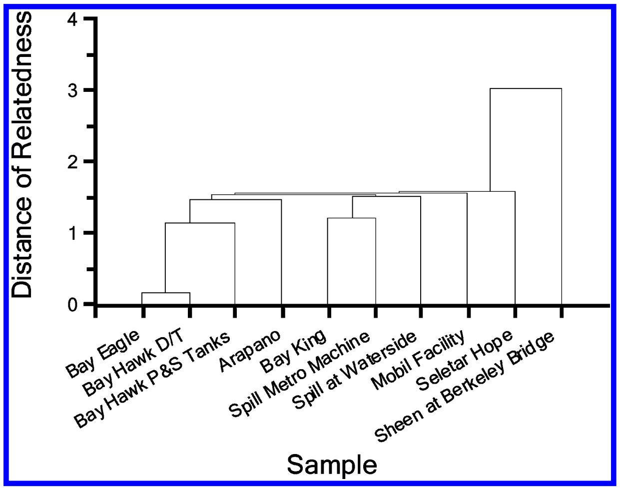

FIGURE 4. Cluster analysis of corrected Norfolk fuel spill samples.

In the present study with fuel oil spill samples, we determined that the *Sheen* at Berkeley Bridge sample was significantly different from the other two spill samples, spill at Metro Machine and spill at waterside. The U.S. Coast Guard analysis suggested that all of these potential source samples were from the same type of fuel oil, but that some weathering did occur before sample collection. Because the *Sheen* at Berkeley Bridge represented the most diffuse and most weathered source in this study, an attempt was made to mathematically correct the measured values with small shifts in isotope values. A figure in Mansuy et al. (*36*) measured the carbon isotope values of unweathered and weathered fuel oils. From their figure (Figure 4), we averaged the small shifts in isotope values from two sources of weathered and unweathered oils using the scale presented. Although our estimates are based soley on the figure, we used the following shifts to "correct" putative weathering in the *Sheen* at Berkeley Bridge sample: C_{12}−0, C_{13}−+0.625, C_{14}−+0.575, C_{15}−+0.325, C_{16}−+0.3, C_{17}−+0.5. While these correction factors are very close to the analytical precision of the IRMS (±0.3‰), they were used to demonstrate the proof of concept.

Once the values were corrected, statistical tests were rerun to determine if new significant differences were obtained for PCA−MANOVA tests, or different relationships evolved from hierarchical clustering analyses. Because PCA assesses variability, there should be no differences when correcting by the addition of constants. Using the putative correction values, a re-run of the PCA−MANOVA test showed the *Alert*

TABLE IV.[a] Results of PCA−MANOVA Significance Tests for Weathering-Corrected Norfolk Spill Samples[a]

sample 1	sample 2	P	capture %	±
"uncorrected values"				
spill Metro Machine	Sheen at Berkeley Bridge	0.004	99.2	0.00003
spill at waterside	Sheen at Berkeley Bridge	0.006	99.6	0.00002
Alert D/T	Sheen at Berkeley Bridge	0.047	95.5	0.00210
Bay Eagle	Sheen at Berkeley Bridge	0.076	96.7	0.00251
Bay Hawk D/T	Sheen at Berkeley Bridge	0.111	97.0	0.00333
"corrected values"				
spill Metro Machine	Sheen at Berkeley Bridge	0.006	98.8	0.00007
spill at Waterside	Sheen at Berkeley Bridge	0.009	99.5	0.00005
Alert D/T	Sheen at Berkeley Bridge	0.083	95.9	0.00339
Bay Eagle	Sheen at Berkeley Bridge	0.111	95.8	0.00463
Bay Hawk D/T	Sheen at Berkeley Bridge	0.160	96.2	0.00609

[a] Results reflect C11 − C17 hydrocarbons.

D/T no longer as a significantly different source than the *Sheen* at Berkeley Bridge ($P = 0.047$ uncorrected; $P = 0.083$ corrected) (Table 4). Although the *Sheen* at Berkeley Bridge was still significantly different from the other spill sources, the corrected P value for comparison to the spill at waterside approached the 1% significance level ($P = 0.009$). When the putative sources identified in the previous section were analyzed by hierarchical clustering with the weathering-corrected *Sheen* at Berkeley Bridge values, the distance of relatedness between the *Sheen* at Berkeley Bridge and other sample clusters is decreased by roughly one arbitrary unit of relatedness (Figure 3B and Figure 4). It is unclear whether the spill at Berkeley Bridge is from the same source spill; however, with modest isotopic corrections for weathering it becomes more related to the other two spill sources and to tank samples from local tugs. Although fractionation of *n*-alkanes due to weathering has been shown to be minimal and essentially within analytical precision of IRMS instrumentation, if fractionation factors are known, correction could be applied before evaluating putative sources with unknown source samples (i.e., spills). This could be done in the initial stages of the proposed source comparison protocol (Figure 2).

Refinements and Applicability. In this work, we utilized existing data sets of stable isotopes collected from a single type of fuel hydrocarbon (diesel). In the years since these data were generated, the science of GC−combustion−IRMS has progressed significantly. With increasing numbers of "certified" standards available from NIST and the Internation Atomic Energy Agency, it is now possible to accurately calibrate each run by co-injecting a certified standard, which is then subject to the same chromatographic and combustion processes as the experimental sample. This allows more accurate reporting of isotope ratios than direct injection of calibration gas standards into the IRMS source (cf., ref *31*). In addition, new software from instrument manufacturers (in our case, Thermo-electron) allows for more sophisticated algorithms for peak detection and background determination.

One factor which may lead to variability in individual component isotope measurements is the presence of an unresolved complex mixture (UCM) found in fuel samples. This high background signal can potentially cause difficulty when trying to separate desired compound isotope values from unresolved compounds. Commercial IRMS instrumentation software can account for a background signal by using the isotopic signal of the preceding nonpeak signal in a chromatogram. Newer software (for example, Thermo Finnigan IsoDAT NT) may provide additional flexibility in removing background signals, although there appears to be limited literature on the application of the different background subtraction algorithms. If the individual compound signal is large, relative to the UCM signal, initial sample cleanup has been shown to be not necessary (*36*). In highly weathered samples (microbial degradation in particular), pretreatment of the sample to minimize the relative contribution of the UCM may be necessary (*36*, *37*). The spill samples used in this work were likely only lightly weathered so no pretreatment was performed. However, the influence of the UCM appears to not have been adequately addressed in the literature. A reasonable follow-on study would be to investigate the ability of IRMS software to accurately account for the UCM by mixing highly weathered UCM with varying concentrations of isotopically known alkane standards. Because all of the sample carbon is combusted to CO_2 before IRMS analysis, per-carbon influences of each fraction could be accounted for by determining the relative peak area of UCM and standard, then calculating the true integrated value using previously reported methods (cf., ref *38*).

Current work in our laboratory is devoted to applying refined analytical techniques to other analytes such as BTEX, fatty acids, and humic material oxidation products. No matter how precise and accurate a data set is, no rigorous and robust statistical method previously existed for evaluating similarity among samples. Because the emphasis of this paper is to define such a statistical technique rather than to concentrate on analytical methodology or site-specific factors, we chose archived data sets processed with relatively antiquated software (by today's standards). In order for the processing routine to work best, the data must conform to the following general guidelines: (1) the data should be systematically comparable, i.e., referenced to the same standard for all replicate measurements. The data need not be referenced to a known calibration standard, only all referenced to the same calibration standard; (2) the data should be as precise as possible, with less than or equal to 1‰ variation between replicate measurements; and (3) at least triplicate measurements should be made as the first three PCs scores from pairwise PCA analysis generally contain greater than 90% ($n = 172$) of the original data variability. This allows for a robust application of MANOVA, giving the true probably of sample relation.

Acknowledgments

The opinions and assertions contained herein are not to be construed as official or reflecting the views of the U.S. Navy or the federal government at large. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. The authors wish to thank the former Fuels Section at NRL for samples, Craney Island Fuel

Depot Quality Control Branch, and the U.S. Coast Guard for samples, collaboration and information.

Supporting Information Available

The first Microsoft Excel spreadsheet (Simulated Data tab) contains the artificial data used to test the statistical processing routine. Two sets of random $\delta^{13}C$ values were produced as described in the green-blocked text (random number generator). The second tab (Tanks) contains the compiled data from the Norfolk tank samples. Each sample has three replicate analyses (Rep in column heading). Values from $C_{10}-C_{17}$ alkanes are presented. The third tab (Norfolk Tugs) contains data from the spill and tug samples from Norfolk, VA. The format is similar to the Tank tab. These data are provided so readers may reproduce our simulation results. A plain text file is available for the Matlab m-file (function) which performs the PCA-MANOVA routine described in the paper. It was uploaded as text file due to selection limitations for uploading. It can be renamed to custom_manova_pca_rep.m and used within Matlab to run the statistical test(s) described herein. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Stout, S. A.; Uhler, A. D.; McCarthy, K. J. A strategy and methodology for defensibly correlating spilled oil to source candidates. *Environ. Forensics* **2001**, *2*, 87−98.

(2) Meglen, R. R. Examining large databases: a chemometric approach using principal component analysis. *Mar. Chem.* **1992**, *39*, 217−237.

(3) ASTM. *Standard practice for oil spill source identification by gas chromatography and positive ion electron impact low resolution mass spectrometry,* D5739−95; ASTM International: West Conshohocken, PA, 1995.

(4) ASTM. *Standard test methods for comparison of waterborne petroleum oils by gas chromatography,* D3328−98; ASTM International: West Conshohocken, PA, 1998.

(5) Frysinger, G. S.; Gaines, R. B.; Xu, L.; Reddy, C. M. Resolving the unresolved complex mixture in petroleum-contaminated sediments. *Environ. Sci. Technol.* **2003**, *37*, 1653−1662.

(6) Reddy, C. M.; Quinn, J. G. GC-MS analysis of total petroleum hydrocarbons and polycyclic aromatic hydrocarbons in seawater samples after the North Cape oil spill. *Mar. Pollut. Bull.* **1999**, *38*, 126−135.

(7) Stout, S. A.; Magar, V. X.; Uhler, R. M.; Ickes, J.; Abbott, J.; Brenner, R. Characterization of naturally occurring and anthropogenic PAHs in urban sediments—Wycoff/Eagle Harbor Superfund site. *Environ. Forensics.* **2001**, *2*, 287−300.

(8) Mudge, S. M. Reassessment of the hydrocarbons in Prince William Sound and the Gulf of Alaska: Identifying the source using partial least-squares. *Environ. Sci. Technol.* **2002**, *36*, 2354−2360.

(9) Christensen, J. H.; Hansen, A. B.; Christensen, J. H.; Mortensen, J.; Andersen, O.; Tomasi, G. Integrated methodology for forensic oil spill identification. *Environ. Sci. Technol.* **2004**, *38*, 2912−2918.

(10) Fry, B. Sources of carbon and sulfur nutrition for consumers in three meromictic lakes in New York State. *Limnol. Oceanogr.* **1986**, *31*, 79−88.

(11) Peterson, B. J.; Fry, B.; Hullar, M.; Saupe, S.; Wright, R. T. The distribution and stable carbon isotopic composition of dissolved organic carbon in estuaries. *Estuaries* **1994**, *17*, 111−121.

(12) Coffin, R. B.; Cifuentes, L. A. Analysis of microbial carbon cycling with stable isotope analysis in the Perdido Estuary, FL. *Estuaries* **1999**, *22*, 917−926.

(13) Meckenstock, R. U.; Griebler, C.; Morasch, B.; Richnow, H. H. Stable isotope fractionation analysis as a tool to monitor biodegradation in contaminated acquifers. *J. Contam. Hydrol.* **2004**, *75*, 215−255.

(14) Murray, A. P.; Edwards, D.; Hope, J. M.; Boreham, C. J.; Booth, W. E.; Alexander, R. A.; Summons, R. E. Carbon isotope biogeochemistry of plant resins and derived hydrocarbons. *Org. Geochem.* **1998**, *29*, 1199−1214.

(15) Kvenvolden, K. A.; Carlson, P. R.; Warden, A.; Threlkeld, C. N. Carbon isotopic comparisons of oil products used in the developmental history of Alaska. *Chem. Geol.* **1998**, *152*, 73−84.

(16) Slater, G. F. Stable isotope forensics—When isotopes work. *Environ. Forensics* **2003**, *4*, 13−23.

(17) O'Malley, V. P.; Abrajano Jr, T. A.; Hellou, J. Determination of the $^{13}C/^{12}C$ ratios of individual PAH from environmental samples: can PAH sources be apportioned. *Geochim. Cosmochim. Acta.* **1994**, *21*, 809−822.

(18) Mazeas, L.; Budzinski, H.; Raymond, N. Absence of stable carbon isotope fractionation of saturated and polycyclic aromatic hydrocarbons during aerobic bacterial biodegradation. *Org. Geochem.* **2002**, *33*, 1259−1272.

(19) Philp, R. P.; Allen, J.; Kuder, T. The use of the isotopic composition of individual compounds for correlating spilled oils and refined products in the environment with suspected sources. *Environ. Forensics* **2002**, *3*, 341−348.

(20) Smallwood, B. J.; Philp, R. P.; Allen, J. D. Stable carbon isotopic composition of gasolines determined by isotope ratio monitoring gas chromatography mass spectrometry. *Org. Geochem.* **2002**, *33*, 149−159.

(21) Kelley, C. A.; Hammer, B. T.; Coffin, R. B. Concentrations and Stable Isotope Values of BTEX in Gasoline-Contaminated Groundwater. *Environ. Sci. Technol.* **1997**, *31*, 2469−2472.

(22) Coffin, R. B.; Miyares, P. H.; Kelley, C. A.; Cifuentes, L. A.; Reynolds, C. M. Stable carbon and nitrogen isotope analysis of TNT: Two- dimensional source identification. *Environ. Toxicol. Chem.* **2001**, *20*, 2676−2680.

(23) Smallwood, B. J.; Philp, R. P.; Burgoyne, T. W.; Allen, J. D. The use of stable isotopes to differentiate specific source markers for MTBE. *Environ. Forensics* **2001**, *2*, 215−221.

(24) Meier-Augenstein, W. The chromatographic side of isotope ratio mass spectrometry − Pitfalls and answers. *LC-GC Eur.* **1997**, *10*, 17−25.

(25) Pemeger, T. V. What's wrong with Bonferroni adjustments. *Brit. Med. J.* **1998**, *316*, 1238.

(26) Läuter, J.; Glimm, E.; Kropf, S. Multivariate tests based on left-spherically distributed linear scores. *Annal Stats.* **1998**, *26*, 1972−1988.

(27) Läuter, J.; Glimm, E.; Kropf, S. New Multivariate Tests for Data with an Inherent Structure. *Biometrics J.* **1996**, *38*, 5−23.

(28) Langsrud, Ø. 50−50 multivariate analysis of variance for collinear responses. *The Statistician.* **2002**, *51*, 305−317.

(29) Malinowski, E. R. *Factor Analysis in Chemistry*, 2nd ed.; John Wiley & Sons: New York. 1991.

(30) Hammer, B. T.; Kelley, C. A.; Coffin, R. B.; Cifuentes, L. A.; Mueller, J. G. $\delta^{13}C$ values of polycyclic aromatic hydrocarbons collected from two creosote-contaminated sites. *Chem. Geol.* **1998**, *152*, 43−58.

(31) Meier-Augenstein, W. Applied gas chromatography coupled-to-isotope ratio mass spectrometry. *J. Chromatogr. A.* **1999**, *842*, 351−371.

(32) Hendrick, M. S. *Oil Spill Identification Report,* Case# MC01000232*;* 01−077; 2001.

(33) Hendrick, M. S. *Oil Spill Identification Report,* Case# MC01000232; 01−079; 2001.

(34) O'Malley, V. P.; Burke, R. A.; Schlotzhauer, W. S. Using GC-MS/Combustion/IRMS to determine the $^{13}C/^{12}C$ ratios of individual hydrocarbons produced from the combustion of biomass materials − application to biomass burning. *Org. Geochem.* **1997**, *27*, 567−581.

(35) Schmidt, T. C.; Zwank, L.; Elsner, M.; Berg, M.; Meckenstock, R. U.; Haderlein, S. B. Compound-specific stable isotope analysis of organic contaminants in natural environments: a critical review of the state of the art, prospects, and future challenges. *Anal. Bioanal. Chem.* **2004**, *378*, 283−300.

(36) Mansuy, L.; Philp, R. P.; Allen, J. Source identification of oil spills based on the isotopic composition of individual components in weathered oil samples. *Environ. Sci. Technol.* **1997**, *31*, 3417−3425.

(37) Pond, K. L.; Huang, Y.; Wang, Y.; Kulpa, C. F. Hydrogen isotopic composition of individual n-alkanes as an intrinsic tracer for bioremediation and source identification of petroleum contamination. *Environ. Sci. Technol.* **2002**, *36*, 724−728.

(38) Goñi, M. A.; Eglinton, T. I. Stable carbon isotopic analyses of lignin-derived CuO oxidation products by isotope ratio monitoring-gas chromatography−mass spectrometry (irm-GC-MS). *Org. Geochem.* **1996**, *24*, 601−615.

Received for review May 23, 2005. Revised manuscript received January 13, 2006. Accepted January 18, 2006.

ES050975P