ASSESSMENT OF HYDROCARBON CARRYOVER POTENTIAL FOR SIX FIELD CLEANING PROTOCOLS

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Assessment of Hydrocarbon Carryover Potential for Six Field Cleaning Protocols

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Executive Summary

Sample collection in the field faces many challenges including, but not limited to, the equipment and materials that can be brought safely into the field. Field teams will often reuse sample collection gear by cleaning it between sampling sites to prevent cross-contamination. Decontamination protocols for equipment used to collect samples for organic contaminant analysis range from a simple water rinse with scrub brush application to more involved measures that include soap and/or the use of various organic solvents. The efficacy of six different field cleaning protocols for sediment sampling tools was evaluated in a controlled laboratory setting using two different sediments (predominantly sand and silt) that were dosed with weathered Deepwater Horizon oil (i.e. “Slick B oil”; collected from surface slicks on June 19, 2010). Percent (%) residual total extractable hydrocarbons (TEH) and percent residual polycyclic aromatic hydrocarbons (PAH) were measured to evaluate the relative effectiveness of the various protocols in removing oiled sediment from the stainless steel materials. Cleaning protocols encompass scenarios where decontamination is limited by resources, supplies, and time, as well as scenarios where resources, supplies, and time are not a factor.

Key findings from this study include:

- The measured % residual TEH and PAH values from all protocols in this study were within the range of values that would be indistinguishable from expected sampling, laboratory, or instrumental variability; thus, regardless of decontamination protocol, all were effective at hydrocarbon removal.
- Cleaning protocols that used organic solvents as a step had less % residual TEH and % residual PAH than protocols that didn’t use solvent as a step.
- The highest % residual TEH and % residual PAH value (0.032% and 0.029% respectively) were obtained from the cleaning protocol that only used site water and a scrub brush.

Introduction

Collecting samples in the field for chemical analysis can be challenging. Sampling efforts are often limited by both the amount of material and equipment (i.e., sampling gear) that can be carried in the field as well as the types of supplies (i.e., soap, solvents) that can be safely transported, handled, and disposed. For example, field teams may not always be able to carry and use solvents safely in the field. With these limitations, field efforts often include sample collection gear that is used multiple times and cleaned (decontaminated) between stations to avoid cross-contamination of samples. Various multi-step field cleaning protocols have been developed and some include the use of organic solvents. It is, therefore, important to understand the risks and benefits of the different cleaning practices.

The National Centers for Coastal Ocean Science (NCCOS), in collaboration with the Office of Response and Restoration (OR&R), developed a testing design to determine if there are measurable chemical residues, related to environmental oiling, in sampling equipment following decontamination. The test design assessed chemical carryover between sediment samples, collected with sampling gear that has been cleaned following several conventional protocols. Six different cleaning protocols were tested (Table 1) on two different sediment types (one predominantly fine silt and the other primarily sand) that were spiked with Slick B oil (a weathered form of Louisiana Sweet Crude oil collected in large quantities from surface slicks on July 19, 2010 during the Deepwater Horizon Oil Spill event). Chemical analyses for total extractable hydrocarbons (TEH) and polycyclic aromatic hydrocarbons (PAHs) were performed to determine residual hydrocarbon contaminants.

Table 1: Cleaning protocols tested in this study.

Cleaning Protocol (CP)	Cleaning Protocol	Protocol Designation
CP1	site water and brush	minimal decontamination (limited resources, supplies, time)
CP2	site water/soap and brush/site water/deionized (DI) water	more rigorous than CP1, for times when solvent is not available or prohibited in the field
CP3	site water and brush/site water/acetone/DI water	NCCOS protocol (Apeti et al., 2012)
CP4	site water/soap and brush/site water/acetone/hexane/air dry	OR&R protocol (Bejarano et al., 2014)
CP5	site water and brush/isopropyl alcohol wipe/DI water	EPA protocol (Fisher et al., 2004)
CP6	site water/soap and brush/site water/isopropyl alcohol wipe/DI water	EPA protocol (Fisher et al., 2004)

Materials and Methods

Sediment Collection

Two different sediment types were collected from estuarine locations in Charleston, SC that have been previously determined to contain low or non-detectable levels of hydrocarbons. Folly River sediment (N32.63898; W-79.98767) is predominantly sand and has been used extensively as a reference sediment for bivalve toxicity testing (Chung, 1999; Key et al., 2007). Leadenwah Creek sediment (N32.6475; W-80.22168) is predominantly silt and serves as a field reference site for crustacean toxicological research (Scott et al., 1999).

Site Water

Seawater, which was used as "site water" for the cleaning protocols, was obtained from the Charleston Harbor, Charleston, SC. Seawater was allowed to settle for at least 72 hr and mechanically filtered to 25 µm (sand filter). Additional mechanical filtration to 10 µm (cartridge filtration) was followed by exposure to a 150W UV sterilizer to limit bacterial growth prior to experimental testing.

Sampling and Decontamination Materials

Stainless steel bowls (4 quart) and 11-inch stainless steel spoons were purchased from Seva Technical Services, Inc. Twelve-inch natural bristle brushes were obtained from Coronado Distribution Company, Inc. Brushes, bowls, and spoons were reused throughout the study across the six cleaning protocols so extra care was taken to eliminate cross-contamination between protocols. After completing each decontamination protocol, standard operating procedures for thorough decontamination of laboratory equipment were followed: all brushes were sonicated in Liquinox® soap (Alconox, Inc.) and water for 30 min, rinsed with hot tap water and deionized (DI) water, air-dried overnight, then solvent rinsed with methanol prior to reuse. Spoons were treated in the same manner, except that they were solvent rinsed with acetone, dichloromethane, and hexane following soap and water sonication. Bowls were scrubbed and washed by hand with Liquinox soap, hot tap water, and DI water, and then solvent rinsed with acetone, dichloromethane, and hexane.

Solvents used for this project were obtained from VWR (Burdick and Jackson dichloromethane and acetone) or Fisher Scientific (n-hexane and methanol). All solvents used were of high purity (≥99.9% purity), suitable for HPLC and GC analysis. Isopropyl alcohol wipes were manufactured by Techspray and contained 99.8% pure isopropyl alcohol. Liquinox soap was used for any cleaning protocol that required soap.

Sediment Grain Size Analysis/Total Organic Carbon Determination

Grain size analysis relating to silt-clay determination was performed according to methods by Plumb (1981). Grain size distribution determination followed methods from Folk (1974), Lewis (1984), and Lewis and McConchie (1994). Total organic carbon (TOC) analysis was performed using the methods of Heiri et al. (2001).

Sediment Spiking

Roughly 10 L of sediment was collected from each site and stored at 4°C for 14 days. Prior to spiking with Slick B oil, each portion of sediment was well mixed by hand using a solvent-cleaned spoon for 10-15 minutes. Approximately 3700 g of wet sediment was weighed into 4-L pre-cleaned glass jars and spiked with Slick B for an expected nominal concentration of 10,000 mg Slick B/kg wet sediment. This mass of sediment was selected so that there was ample sediment available to aliquot into multiple replicates for each cleaning protocol. The nominal concentration of 10,000 mg Slick B/kg wet sediment was selected to represent a reasonable worst-case field scenario for encountering oil-contamination in soil and sediment, based on a summary review of PAH50 concentrations in thousands of such samples collected during the Deepwater Horizon oil spill natural resource damage assessment (Rouhani et al 2016). Calculated nominal wet mass concentrations were 9,930 and 10,230 mg Slick B/kg wet sediment for Folly River (sand) and Leadenwah Creek (silt) sediment respectively. After spiking, each jar was rolled on a mechanical jar roller for at least 8 hours. After spiking and mixing, the sediment was stored in the dark at 4°C.

Assessment of Cleaning Protocol Carryover Potential

To assess the potential carryover associated with each cleaning protocol, the glass jars of spiked sediment were retrieved from storage, allowed to come to room temperature, and homogenized by a mechanical roller for one hour. For each sample replicate, roughly 500 g of sediment was placed into a stainless steel bowl and stirred with a stainless steel spoon for ~ 30 seconds. The sediment was then scooped by spoon from the bowl after which the bowl and spoon were cleaned according to each designated protocol. For any given protocol, both sediment types were tested (three replicates per sediment type). After the cleaning protocol was completed for each replicate, the bowl was rinsed with approximately 50 mL of dichloromethane and 50 mL of hexane and the spoon was rinsed with approximately 25 mL of dichloromethane and 25 mL of hexane in order to collect and measure any hydrocarbon residues remaining after the completed cleaning protocol. For each replicate, the solvents used to rinse the bowl and spoon were collected into pre-cleaned 500-mL wide-mouth amber bottles.

Measurement of Hydrocarbons in Cleaning Protocol Rinses

The composited dichloromethane and hexane rinses were spiked with isotopically labeled PAH and TEH internal standards (Cambridge Isotope Laboratories), treated with anhydrous sodium sulfate for water removal, concentrated, and processed through silica solid phase extraction (SPE) prior to instrumental analysis on GC/MS.

Measurement of Hydrocarbons in Sediment

Three 10-g aliquots of each sediment type were assessed for PAHs and TEH. Sediment aliquots were extracted using methods adapted from Kucklick et al. (1997), Long et al. (1998), and Balthis et al. (2012). Briefly, sediment was placed into a glass mortar bowl containing anhydrous sodium sulfate to dry the sediment. The sediment and sodium sulfate mixture was transferred to a stainless steel Accelerated Solvent Extraction (ASE) cell, spiked with isotopically labeled PAH and TEH internal standards, and extracted with a mixture of 1:1 dichloromethane:acetone using an ASE 200 system (Dionex). Afterwards the extract was treated with activated copper wool for sulfur removal and passed through additional sodium sulfate for residual water removal. Final extract cleanup steps included gel permeation chromatography (GPC) followed by silica SPE.

Measurement of Hydrocarbons in Seawater and Solvents

The seawater used during the testing, along with the solvents (hexane and dichloromethane) used to rinse the bowl and spoon after each cleaning protocol, were assessed for their potential contribution of hydrocarbons. Three replicates of seawater (each 1 L) were extracted using liquid/liquid extraction. Each seawater sample was sequentially extracted using dichloromethane (60 mL), 1:1 dichloromethane:hexane (60 mL), and hexane (60 mL). In total, 180 mL of solvent was used to extract the seawater. Seawater solvent extracts were combined, then processed through sodium sulfate to remove residual water, concentrated, and cleaned-up with silica SPE. To determine hydrocarbons related to the solvent and/or concentration process, three 150-mL replicates of 1:1 dichloromethane:hexane were concentrated in a water bath (40°C) under nitrogen (N_2), and then passed through silica SPE. Solvent (150 mL of 1:1 dichloromethane:hexane) was also concentrated using a water bath only (no N_2) to determine residual hydrocarbons solely related to the solvent.

Instrumental Analysis

Extracts from all chemical analyses were analyzed using an Agilent GC/MS (6890/5973, electron impact configuration) operated in selected ion monitoring mode, containing a split/splitless injector and DB17ms analytical column (Agilent J&W 60 m x 0.25 mm x 0.25 µm). The instrument was calibrated with calibration standards ranging from 0.1-5000 ng (PAHs) and 0.25-20 mg (TEH). The TEH calibration curve was made by diluting Slick B material and extracting the calibration levels through silica SPE prior to instrumental analysis. Continuing calibration verification standards were run every 10-15 samples to ensure the validity of the calibration curve. All analytes had a coefficient of determination (r^2) greater than or equal to 0.995. Data analysis was performed using MSD Chemstation software. Total PAH (PAH50) is reported for 50 parent and alkylated PAHs (Table 2).

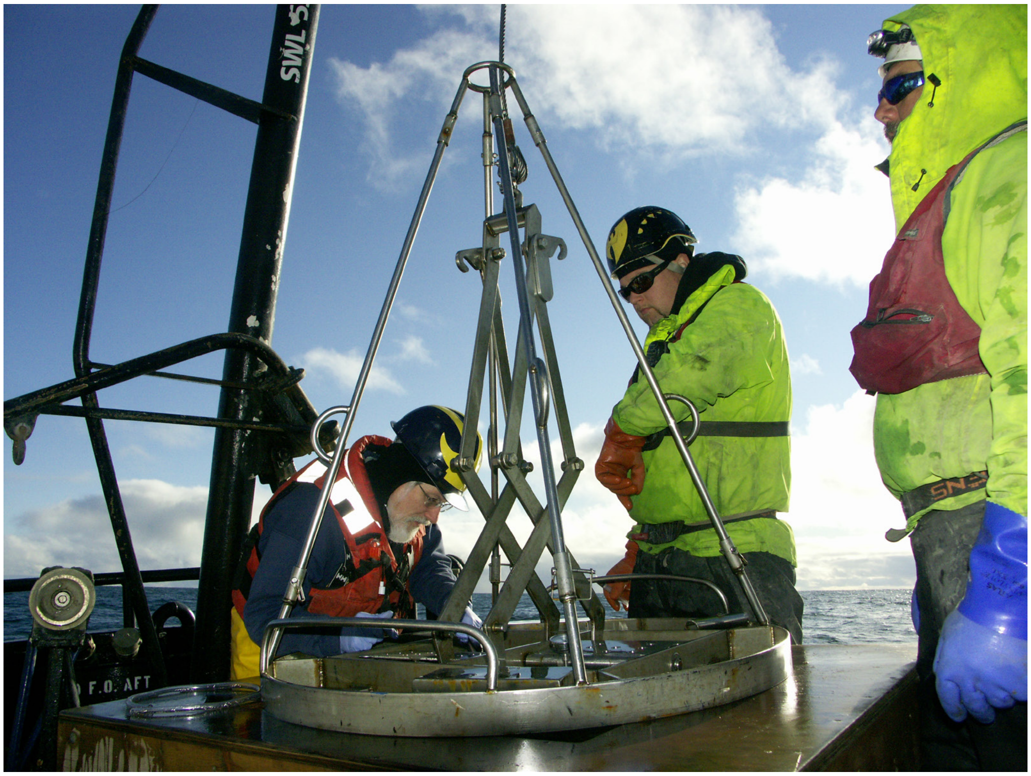


Table 2: Parent and alkylated PAHs that comprise PAH50.

Parent PAHs in PAH50	Alkylated PAHs in PAH50
naphthalene	C1-naphthalenes
biphenyl	C2-naphthalenes
acenaphthene	C3-naphthalenes
acenaphthylene	C4-naphthalenes
fluorene	C1-fluorenes
dibenzofuran	C2-fluorenes
dibenzothiophene	C3-fluorenes
phenanthrene	C1-dibenzothiophenes
anthracene	C2-dibenzothiophenes
fluoranthene	C3-dibenzothiophenes
pyrene	C4-dibenzothiophenes
benz(a)anthracene	C1-phenanthrenes/anthracenes
benzo(b)naphtho(2,1-d)thiophene	C2-phenanthrenes/anthracenes
chrysene + triphenylene	C3-phenanthrenes/anthracenes
benzo(a)fluoranthene	C4-phenanthrenes/anthracenes
benzo(b)fluoranthene	C1-fluoranthenes/pyrenes
benzo(j)fluoranthene	C2-fluoranthenes/pyrenes
benzo(k)fluoranthene	C3-fluoranthenes/pyrenes
benzo(a)pyrene	C4-fluoranthenes/pyrenes
benzo(e)pyrene	C1-chrysenes/benzanthracenes
dibenzo(a,h)anthracene	C2-chrysenes/benzanthracenes
indeno(1,2,3-c,d)pyrene	C3-chrysenes/benzanthracenes
benzo(g,h,i)perylene	C4-chrysenes/benzanthracenes
	C1-naphthobenzothiophenes
	C2-naphthobenzothiophenes
	C3-naphthobenzothiophenes
	C4-naphthobenzothiophenes

Method Detection Limits and Blank Correction

Method detection limits (MDLs) for TEH were calculated by using the lowest calibration point (0.25 mg) and dividing that by the mass or volume of sample in the extract. The MDLs for PAHs were calculated according to Ragland et al. (2014). Briefly, the minimum detectable peak area (MDPA) of any given analyte was estimated using procedural blanks by taking into account instrument sensitivity, as well as extraction efficiency, relating to both the process and analyst (Ragland et al., 2014). The general equation for calculating the PAH MDLs can be found in Supplemental Figure 1. The procedural blanks used for calculating the MDLs were obtained from the solvent (with N_2 concentration) extracts.

For each cleaning protocol, a blank (a spoon and bowl, without any sediment) was processed using the same cleaning protocol as the bowls and spoons that contained sediment. These process blanks were used to understand if and how the cleaning protocol itself contributed to possible PAH/TEH concentrations. Process blanks were run at the end of each cleaning protocol. Any measured PAHs found in a given process blank were subtracted from measured PAHs observed in replicate samples from the same cleaning protocol.

Statistical Analysis and Units of Measurement

All statistical analyses were performed using JMP version 12.1.0. All datasets were tested for normality prior to statistical analyses. If a dataset had a p-value greater than 0.05, it passed the test for normality, whereas datasets with a p-value less than or equal to 0.05 failed the test for normality. For datasets with normal distributions, the Student's t-test or a one-way analysis of variance (ANOVA) with a Tukey-Kramer pairwise comparison (when applicable) was used to examine differences between treatments. For datasets with non-normal distributions, equivalent non-parametric statistical analyses (Wilcoxon test or Kruskal-Wallis test with the Dunn Method for joint ranking) were used.

Due to large differences in scale between TEH and PAH50, TEH is reported in units of mg, mg/kg, mg/L or ppm while PAH50 is reported in units of ng, ng/g, ng/mL, or ppb.

Results and Discussion

Grain Size and Total Organic Carbon Analysis

The quantified grain size and TOC confirmed that the two sediment sampling locations represented two different sediment types. In this study, having different sediment types was important in understanding how factors such as grain size and/or TOC may influence potential routes of cross-contamination. Generally, organic contaminants with a high log K_{ow} tend to preferentially bind to silt/clay based sediments, which typically have higher TOCs. Sediment from the Folly River was predominantly sand (93.8%; Supplemental Table 1) while the Leadenwah Creek sediment was 48.8% sand (Supplemental Table 1). Percent TOC for Folly River and Leadenwah Creek was measured as 1.27±0.04% and 7.92±0.05%, respectively.

Hydrocarbons in Seawater and Solvent

Results for the seawater and solvent replicates are shown in Table 3. In both seawater and solvent (with and without N_2 concentration) TEH was below the method detection limit (0.0003-0.0017 mg/mL). There were measurable PAHs in both seawater and N_2 concentrated solvent. There were no PAHs detected in the solvent where N_2 concentration was not used. PAH50 for the seawater and N_2 concentrated solvent was normalized to the volume of solvent used and compared using a t-test. There was no significant difference between the seawater and N_2 concentrated solvent PAH50 residues (p=0.0862) when normalized to solvent volume. This suggests that measured PAHs were more likely related to the extraction process (i.e., N_2 gas concentration step) rather than the seawater and/or solvent.

Naphthalene, 1-methylnaphthalene, and 2-methylnapthalene accounted for 90-95% of PAHs that were measured in both seawater and N_2 concentrated solvent (Supplemental Figure 3). These PAHs are some of the more volatile PAHs and appear to be related to the N_2 gas that was used during the concentration steps in the extraction/clean-up process. It should also be noted that extraction solvent volume, which dictates concentration time, appears to affect detected PAH masses. In Table 3, it was observed that the average PAH50 mass for seawater was 102 ng and for solvent alone was 67.1 ng. Corresponding extraction solvent volumes were 200 and 150 mL respectively. Thus, it can be inferred that larger solvent volumes, which require longer N_2 evaporation time, lead to higher detected hydrocarbon masses. These analytes at these reported concentrations are typical for our laboratory processes. Efforts were made to limit hydrocarbon contamination by using an in-line filter designed to remove hydrocarbons on the nitrogen gas line. Despite this effort, hydrocarbon contamination was observed and therefore measured PAHs in the solvent were factored into the calculated method detection limits (MDL).

Table 3: Measured TEH and PAH50 from Charleston Harbor seawater and extraction solvents (with and without N_2 concentration). PAH50 measurements are given in mass of PAHs measured (which does not take extraction volume nor solvent volume used into account), PAH50 concentration based on the amount of seawater extracted (only applicable to seawater samples), and PAH50 concentration based on the volume of solvent used.

	TEH (mg)	PAH50 (ng)	PAH50 conc. based on water vol. (ng/mL)	PAH50 conc. based on solvent vol. (ng/mL)
Seawater 1	0.00	93.4	0.0934	0.467
Seawater 2	0.00	114	0.114	0.570
Seawater 3	0.00	99.4	0.0994	0.497
Average	--	*102*	*0.102*	*0.511*
(Std. Dev)	--	*(10.6)*	*(0.011)*	*(0.053)*
Solvent with N_2 1	0.00	56.9	--	0.379
Solvent with N_2 2	0.00	67.8	--	0.452
Solvent with N_2 3	0.00	66.4	--	0.443
Average	--	*67.1*	--	*0.425*
(Std. Dev)	--	*(0.99)*	--	*(0.040)*
Solvent (no N_2) 1	0.00	0.00	--	0.00
Solvent (no N_2) 2	0.00	0.00	--	0.00
Solvent (no N_2) 3	0.00	0.00	--	0.00

Hydrocarbons in Process Blanks

The measured TEH and PAH50 values from the process (i.e., cleaning protocol) blanks are shown in Table 4. Measured TEH was below the detection limit for all cleaning protocols except for CP5. Measured PAH50 concentration based on solvent volume for the process blanks (Table 4), N_2 concentrated solvent, and seawater (Table 3) were compared using an ANOVA. A significant difference was observed among treatments (p=0.0277) and a Tukey-Kramer pairwise comparison revealed that PAH50 concentrations in the blank were greater than PAH50 concentrations in the solvent (p=0.0346). There was no significant difference between the process blanks and seawater (p=0.1107). Naphthalene, 1-methylnaphthalene and 2-methylnapthalene account for 61-83% of PAHs that were measured in the process blanks (Supplemental Figures 2A-B, 3). Observationally, there were decreases in the proportions of the naphthalenes between the solvent/seawater and the cleaning protocol blanks which may indicate that the measured PAHs are not solely related to the laboratory extraction/cleanup process (i.e., nitrogen gas) but that there is also a minor contribution from the cleaning protocol. In protocols where soap and water were required, only one soap and water bath was made and used throughout that cleaning protocol, which may be a potential source of cross-contamination in samples and ultimately the blanks. The decision was made to use the measured PAHs from the process blanks to correct samples from the corresponding cleaning protocol. Measured values for any detected PAH analyte found in the blanks were subtracted from the associated PAHs in the individual replicates for a given cleaning protocol. This correction was made in order to account for hydrocarbon contributions occurring from the extraction process as well as from the soap and water bath.

Table 4: Measured TEH and PAH50 for the blanks associated with each cleaning protocol.

Cleaning Protocol	TEH (mg)	PAH50 (ng)	PAH50 conc. based on solvent volume (ng/mL)
CP1	<MDL	127	0.747
CP2	<MDL	150	0.882
CP3	<MDL	102	0.600
CP4	<MDL	141	0.829
CP5	0.32	187	1.10
CP6	<MDL	83.4	0.491

Hydrocarbons in Spiked Sediment

Measured TEH and PAH50 for the spiked sediment are found in Table 5. The targeted Slick B whole oil concentration in the sediment was 10,000 mg/kg wet. Average measured TEH in the spiked sand (Folly) was 8930±780 mg/kg wet and in the spiked silt (Leadenwah) was 9350±1470 mg/kg wet. On a dry mass basis TEH was 12600±1100 and 24500±3900 mg/kg respectively. Average PAH50 was 27600±1150 and 28600±5410 ng/g wet for the sand and silt sediments respectively (on a dry mass basis PAH50 was 38800±1620 and 75000±14200 ng/g respectively). On average, PAH50 accounted for 0.31±0.012% of the total extractable hydrocarbons in Slick B.

Table 5: TEH and PAH50 concentrations from spiked sediments.

Sediment Type (Dry Fraction)	Replicate	TEH mg/kg wet (ppm)	TEH mg/kg dry (ppm)	PAH50 ng/g wet (ppb)	PAH50 ng/g dry (ppb)
Folly River - Sand (0.711)	1	9670	13600	28800	40500
	2	8120	11400	26500	37200
	3	8990	12600	27500	38700
	Average	8930	12600	27600	38800
	(std. dev.)	(780)	(1100)	(1150)	(1620)
Leadenwah Creek - Silt (0.382)	1	10500	27600	33500	87800
	2	9820	25700	29600	77500
	3	7700	20200	22800	59800
	Average	9350	24500	28600	75000
	(std. dev.)	(1470)	(3900)	(5410)	(14200)

Residual Hydrocarbons from Cleaning Protocols

Measured TEH and PAH50 values for each cleaning protocol are found in Table 6. To understand the percentage of TEH and PAH50 that remained in the bowl after the cleaning protocol, the percent (%) residual TEH and % residual PAH50 were calculated. These values were calculated by taking the average TEH and PAH50 wet concentrations for each sediment type (Table 5) and multiplying that by the amount of sediment that was placed in each bowl; this value is the total TEH and PAH50 that was transferred into each bowl. The TEH (or PAH50) residue that was measured after the cleaning process was then divided by the total TEH (or PAH50) and multiplied by 100 to obtain the % residual TEH (or % residual PAH50) for any given replicate in any given cleaning protocol. The % residual TEH and PAH50 generally agreed with each other which indicates that the designated cleaning protocol did not preferentially remove aliphatic hydrocarbons (TEH) over aromatic hydrocarbons (PAH50) or vice versa.

The highest percentage of residual TEH and PAH50 was observed in CP1, silt sediment, replicate 3. Residual TEH and PAH50 for this sample was 0.032% and 0.029% respectively. Within many of the protocols, TEH was less than the detection limit. The lowest recorded % residual PAH50 was observed in CP 4, with 0.0001% residual PAH50. Generally the mass of residual hydrocarbons remaining on the equipment after cleaning was near or below 0.01% (i.e., 0.0001 as a fraction) of the hydrocarbons in the sample material. Thus if reusing the cleaned sampling equipment for a subsequent collection of a sample having similar hydrocarbon contamination, the most additional hydrocarbon contamination the cleaned equipment theoretically could contribute to the new sample is on the order of 0.01%, a negligible difference given the other variability in field sampling and laboratory analysis. Even if the subsequent sample is far less contaminated (e.g., contains two orders of magnitude less TEH or PAH50), the most contamination that could be contributed by the decontaminated equipment would only increase the hydrocarbon concentration in the sample by 1%. It is also important to note that this assumes that all of the residual hydrocarbons would be transferred into the next sample; in a real world scenario this would be highly unlikely. For example, when using a grab sampler, it is common practice to scoop sediment only from the interior of the grab (i.e., sediment that does not touch the sampler itself) thus the potential for residual hydrocarbon contamination in Table 6 may be overestimated.

Table 6: Measured TEH and PAH50 from each bowl/spoon after each cleaning protocol. The % residual TEH and % residual PAH were calculated by multiplying the average TEH (or PAH50) concentration (Table 5) by the amount of sediment placed into each bowl to obtain an estimate of the total TEH (or PAH50) for any given treatment. The measured TEH (or PAH50) measured after each cleaning protocol replicate was divided by the total TEH (or PAH50) and multiplied by 100 to determine % residual TEH (or PAH50).

Cleaning Protocol	*Sediment Type*	*TEH (mg)*	*PAH50 (ng)*	*% Residual TEH*	*% Residual PAH50*
CP1-site water and brush	Sand	1.01	2550	0.020	0.017
		0.760	1930	0.015	0.013
		0.810	1910	0.018	0.013
	Average (Std. Deviation)	*0.860 (0.132)*	*2130 (363)*	*0.018 (0.003)*	*0.014 (0.002)*
	Silt	0.500	1240	0.011	0.009
		0.810	1210	0.017	0.008
		1.53	4220	0.032	0.029
	Average (Std. Deviation)	*0.947 (0.528)*	*2220 (172)*	*0.02 (0.010)*	*0.015 (0.011)*
CP2-site water/ soap and brush/site water/DI	Sand	0.290	716	0.006	0.005
		0.760	978	0.017	0.007
		0.760	1600	0.017	0.013
	Average (Std. Deviation)	*0.603 (0.271)*	*1098 (454)*	*0.013 (0.006)*	*0.008 (0.004)*
	Silt	0.270	797	0.006	0.005
		0.000	514	0.000	0.004
		0.000	342	0.000	0.002
	Average (Std. Deviation)	*0.090 (0.156)*	*551 (230)*	*0.002 (0.003)*	*0.004 (0.002)*
CP3-site water and brush/site water/ acetone/DI	Sand	0.000	74.1	0.000	0.0005
		0.680	249	0.015	0.002
		0.000	123	0.000	0.0009
	Average (Std. Deviation)	*0.227 (0.392)*	*149 (90)*	*0.005 (0.008)*	*0.001 (0.001)*
	Silt	0.620	749	0.013	0.005
		0.310	103	0.006	0.0007
		0.000	159	0.000	0.001
	Average (Std. Deviation)	*0.31 (0.31)*	*337 (358)*	*0.006 (0.006)*	*0.002 (0.002)*
CP4-site water/ soap and brush/ site water/acetone/ hexane/air dry	Sand	0.000	16.0	0.000	0.0001
		0.000	29.2	0.000	0.0002
		0.000	73.1	0.000	0.0005
		0.000 (0.000)	*39.4 (29.8)*	*0.000 (0.000)*	*0.0003 (0.0002)*
	Silt	0.000	20.2	0.000	0.0001
		0.820	894	0.017	0.006
		0.330	331	0.007	0.002
	Average (Std. Deviation)	*0.383 (0.412)*	*415 (443)*	*0.008 (0.008)*	*0.003 (0.003)*
CP5-site water and brush/IPA wipe/DI	Sand	0.000	223	0.000	0.002
		0.000	115	0.000	0.0008
		0.000	256	0.000	0.002
	Average (Std. Deviation)	*0.000 (0.000)*	*198 (744)*	*0.000 (0.000)*	*0.002 (0.001)*
	Silt	0.000	219	0.000	0.002
		0.340	695	0.007	0.005
		0.560	496	0.012	0.003
	Average (Std. Deviation)	*0.300 (0.282)*	*470 (239)*	*0.006 (0.006)*	*0.003 (0.001)*
CP6-site water/ soap and brush/site water/IPA wipe/DI	Sand	0.000	48.8	0.000	0.0003
		0.000	31.2	0.000	0.0002
		0.000	66.9	0.000	0.0005
	Average (Std. Deviation)	*0.000 (0.000)*	*49.0 (18)*	*0.000 (0.000)*	*0.0003 (0.0002)*
	Silt	0.000	77.1	0.000	0.0005
		0.280	254	0.006	0.002
		0.330	328	0.007	0.002
	Average (Std. Deviation)	*0.203 (0.177)*	*220 (128)*	*0.004 (0.003)*	*0.002 (0.001)*

Silt versus Sand Sediment Comparison

To understand if there is a difference between % residual TEH or PAH50 carryover based on sediment type within any given cleaning protocol, the t-test was used (Table 7). There were no significant differences found for any protocol between sediment types for PAH50. For TEH, the only significant difference observed between sediment types was found in cleaning protocol 2 ($p=0.0353$). Based on these observations, the decision was made to pool all replicates within a cleaning protocol, regardless of sediment type, for further analyses.

Table 7: T-test results for silt versus sand sediment comparison within each protocol. "" denotes a significant difference ($p<0.05$) between sediment types for a particular cleaning protocol.*

Cleaning Protocol	TEH	PAH50
CP1		
CP2	*	
CP3		
CP4		
CP5		
CP6		

Protocol Comparisons (using pooled sediment data)

In order to determine if there were differences in the efficacy of each protocol, an ANOVA was performed using log transformed % residual PAH50 data. For this analysis, data from the two different sediment types within a cleaning protocol were pooled (i.e., the three silt and three sand replicates performed in any given cleaning protocol were pooled together). The ANOVA indicated that there was a significant difference among cleaning protocol means ($p<0.0001$). In subsequent Tukey-Kramer pairwise comparisons, significant differences were observed between CP1 and CP3, CP1 and CP4, CP1 and CP5, CP1 and CP6, CP2 and CP4, and CP2 and CP6 (Table 8). A nonparametric Kruskal-Wallis test was performed with the % residual TEH data. The Kruskal-Wallis test showed that there were significant differences among the cleaning protocol means ($p = 0.0132$). A nonparametric comparison for all pairs using the Dunn Method for joint ranking was conducted; significant differences were observed between CP1 and CP5, and CP1 and CP6 (Table 8). From these results it can be inferred that protocols that use solvent as a step (CPs 3-6) were more effective in removing chemical residue than protocols without solvent (CP1 and CP2). This hypothesis was tested for both the % residual PAH50 and % residual TEH data. It was found that there was a significant difference between protocols using solvents versus protocols not using solvent for both the PAH50 and TEH data (Student's t-test, $p<0.0001$ and nonparametric Wilcoxon test, $p=0.0018$ respectively).

Table 8: Results of Tukey-Kramer pairwise (PAH50) and Dunn All Pairs for Joint Ranks (TEH) for comparisons among cleaning protocols using pooled sediment types. "" denotes a significant difference for PAH50, "+" denotes a significant difference for TEH.*

	CP1	CP2	CP3	CP4	CP5	CP6
CP1			*	*	*	*
CP2				*		*
CP3						
CP4						
CP5	+					
CP6	+					

Protocol Comparisons (within sediment types)

Differences between cleaning protocols within each sediment type were also evaluated. To determine if there were significant differences among cleaning protocols for the sand sediment (Folly River sediment), an ANOVA using log transformed % residual PAH50 data was conducted with a follow-up Tukey-Kramer pairwise comparison (Table 9). For the sand sediment, a significant difference was observed among cleaning protocol means ($p<0.0001$). Treatments that were significantly different from one another are denoted by "*" in Table 9. A similar ANOVA was also performed for silt sediment (Leadenwah Creek sediment); there were no significant differences among cleaning protocol means ($p=0.1307$ for % residual PAH50 and $p=0.0968$ for % residual TEH). For the sand sediment, more than half of the % residual TEH data was below the detection limit so there was no statistical analysis applied.

Table 9: Results of Tukey-Kramer pairwise comparisons among cleaning protocols using sand sediment type only (there were no significant differences for silt-PAH50 or silt-TEH). "" denotes a significant difference for PAH50 in sand sediments. Sand-TEH was not performed due more than half of the data being less than the detection limit.*

	CP1	CP2	CP3	CP4	CP5	CP6
CP1			*	*	*	*
CP2			*	*	*	*
CP3						
CP4					*	
CP5						*
CP6						

Results of comparisons among cleaning protocols with the used pooled sediment (Table 8) and those based on sediment type (Table 9) suggest that differences in the effectiveness of different cleaning protocols are driven by sediment type. There were no significant differences related to % residual PAH50 among cleaning protocols for the silt sediment. Conversely, significant differences were observed among cleaning protocols for the sand sediment. A plot of % residual PAH50 by sediment type and cleaning protocol (Figure 1), illustrates that silt replicates encompassed a large amount of variability, while PAH50 measured in the sand replicates was much less variable. The large variability within silt replicates could explain why we were not able to detect statistically significant differences between sediment types within a cleaning protocol and it may also explain why no differences were detected when looking at only the silt sediment across all cleaning protocols. During the cleaning protocol process it was documented (Figures 2A-D) that some of the silt replicates still had visible sediment or oil on the bowl and/or spoon after the initial site water rinse was performed whereas the sand replicates generally appeared to be "clean" after the first rinse (Figure 3). The inherent "sticky" nature of the silt sediment appears to lead to an inconsistent ability to clean the bowl/spoon effectively, which may be responsible for the observed variability. Sand, in contrast, was easier to remove from the bowl and spoon, which led to less variable results.

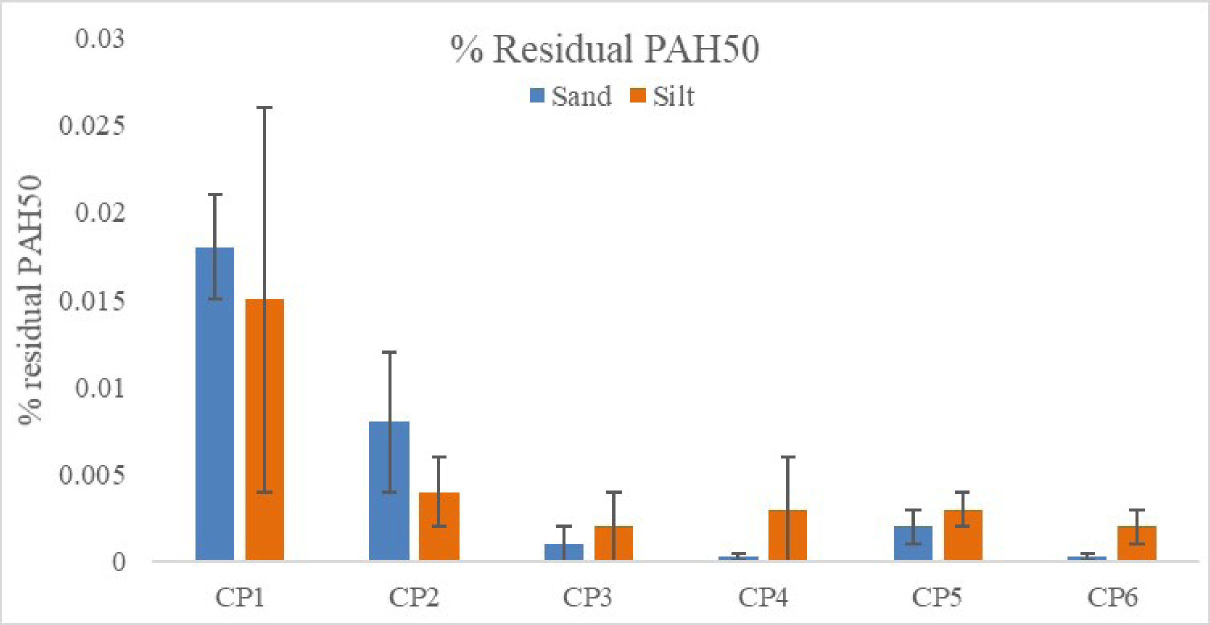


Figure 1: Percent residual PAH50 plotted for each sediment type. Error bars are one standard deviation.

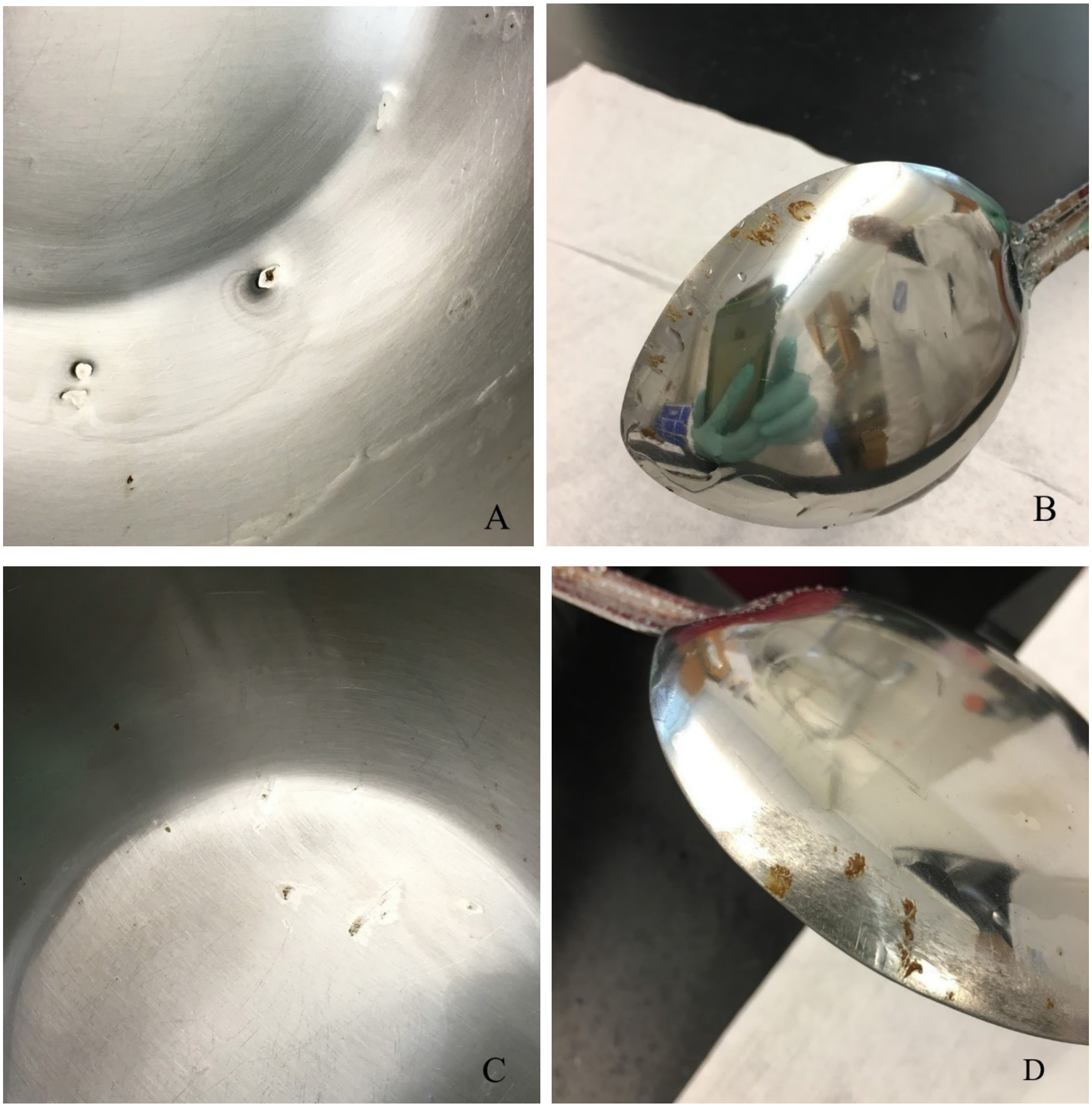


Figure 2A-D: Photos taken during the cleaning protocol process. All photos are from silt replicates and show sediment and/or oil that was remaining after the initial site water rinse. Figure A was from CP2, rep 1 (site water/soap and brush/site water/DI water); B was from CP2, rep 3; C was from CP3, rep 3 (site water and brush/site water/acetone/DI water); and D was from CP4, rep 3 (site water/soap and brush/site water/acetone/hexane/air dry).

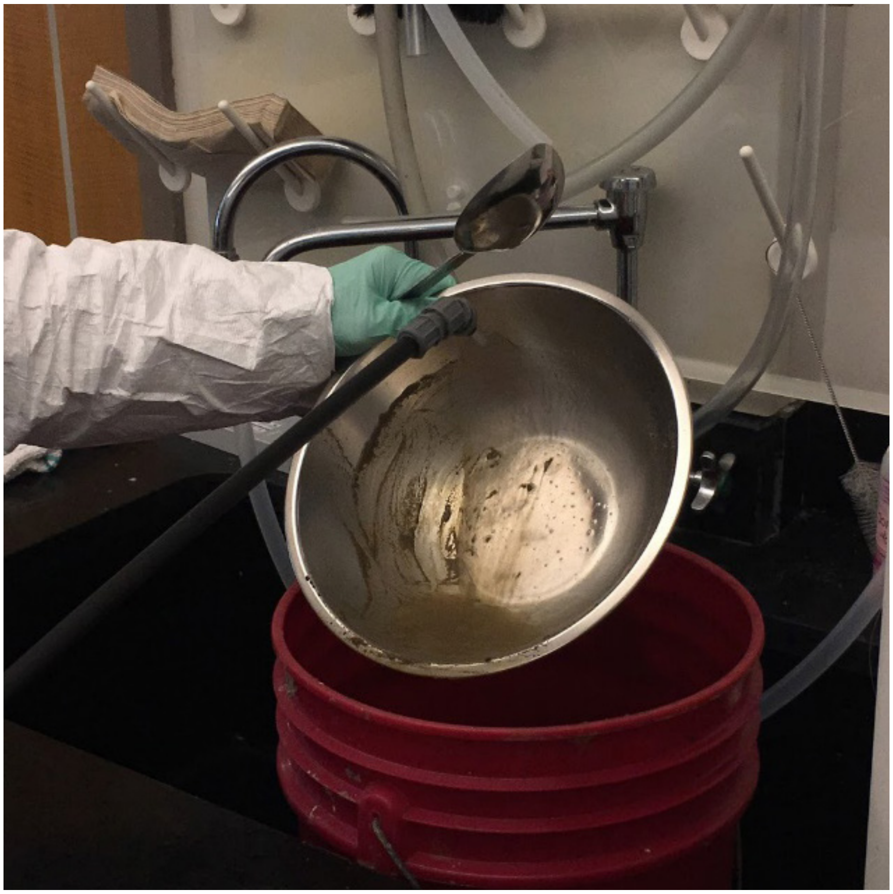


Figure 3: Photo of the site water rinse process during a sand replicate.

The PAH50 profiles, on an average proportional basis, were plotted for each sediment type (Figure 4). PAH profiles between the two sediment types are very consistent. The predominant PAHs in the Slick B spiked sediment were C1-C4 phenanthrenes, C1-C4 dibenzothiophenes and C1-C3 fluorenes. Average residual PAH50 profiles were plotted for each cleaning protocol as well (Figure 5A and 5B). The predominant PAHs that remained after each cleaning protocol were also the C1-C4 phenanthrenes, C1-C4 dibenzothiophenes and C1-C3 fluorenes. Patterns between each protocol are generally consistent. This indicates that certain PAHs are not being selectively removed based on a particular cleaning protocol and also demonstrates that the residual PAHs are related to residual oil rather than laboratory processes as noted earlier.

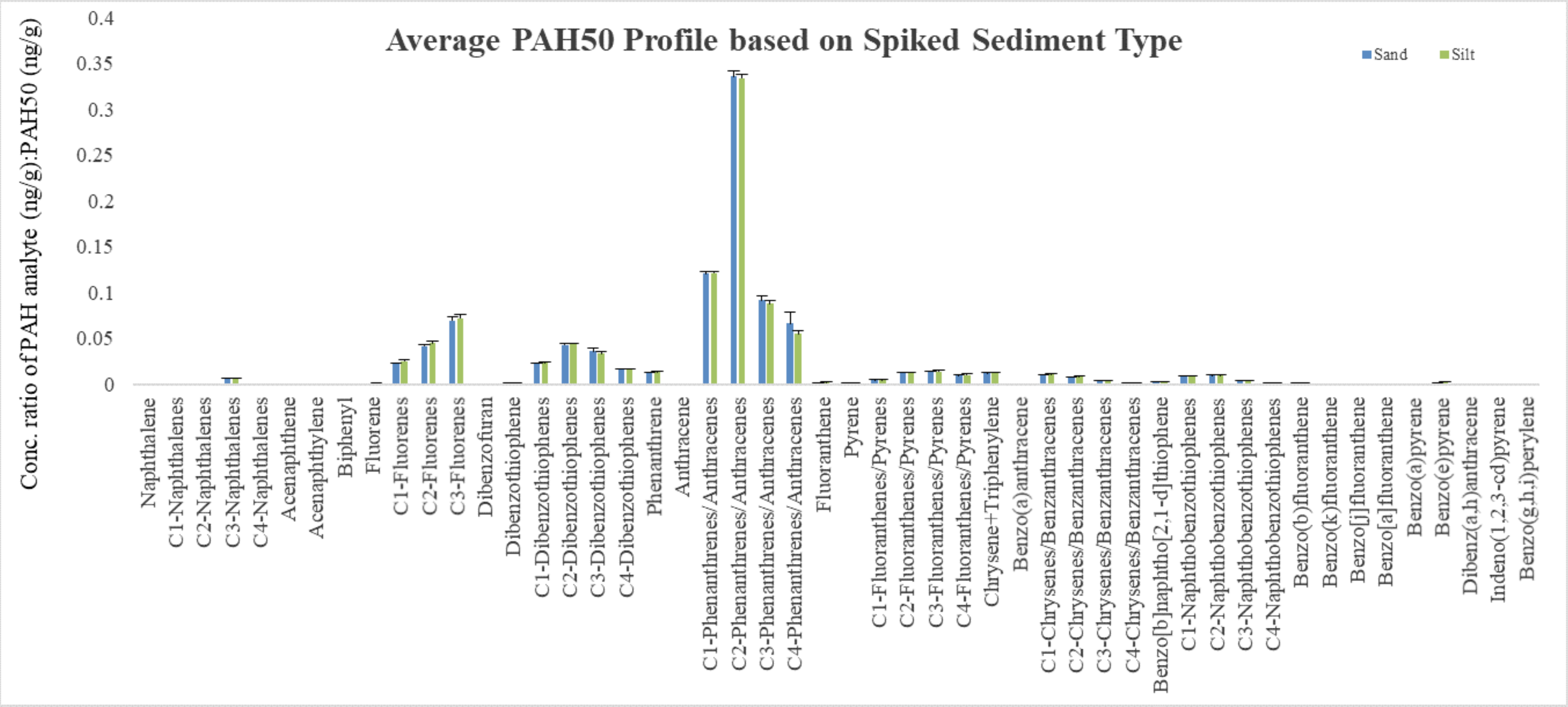
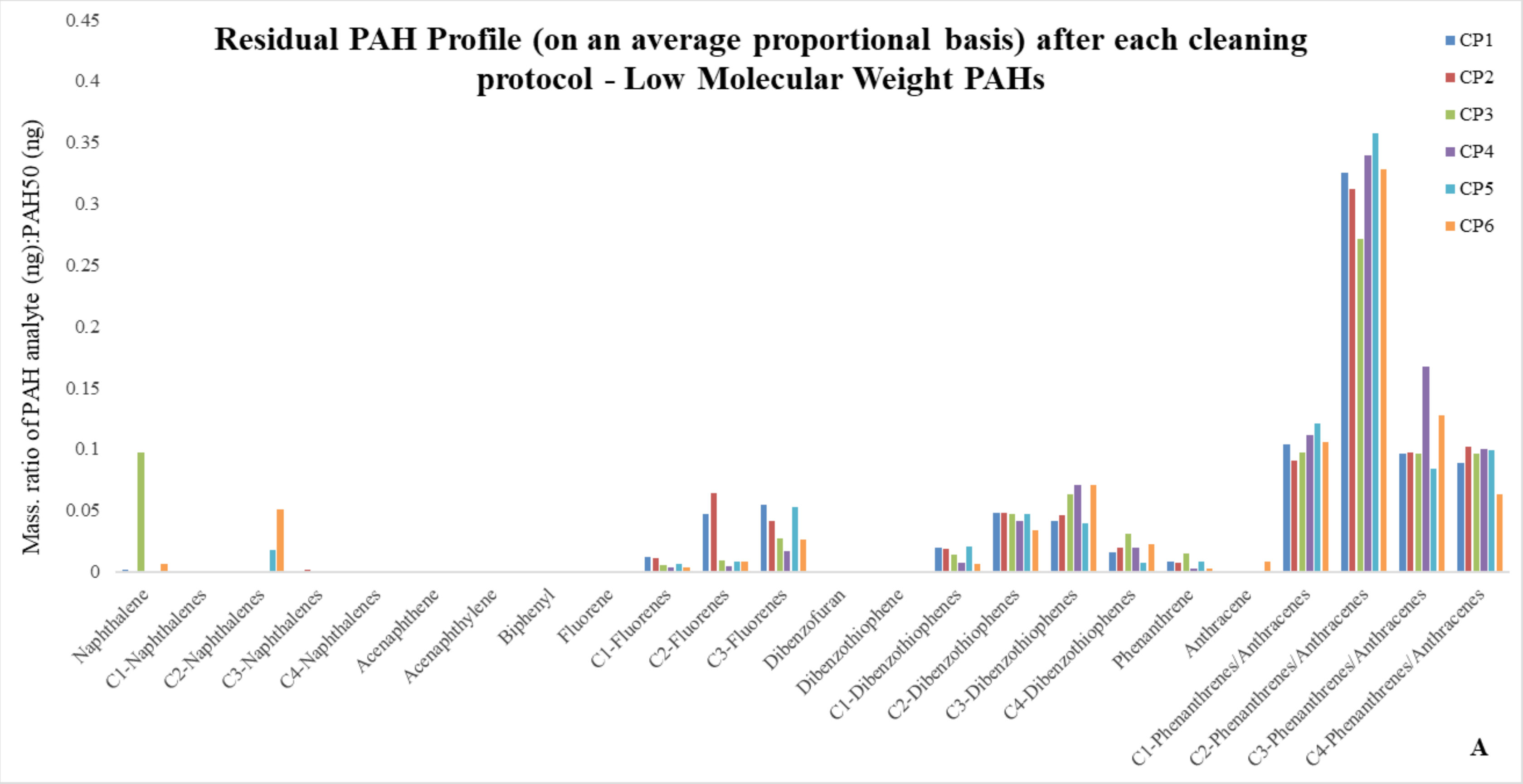


Figure 4: Average PAH50 profiles for both the sand and silt spiked sediments. Error bars are one standard deviation.


Residual PAH Profile (on an average proportional basis) after each cleaning protocol - Low Molecular Weight PAHs
Mass. ratio of PAH analyte (ng):PAH50 (ng)
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Naphthalene
C1-Naphthalenes
C2-Naphthalenes
C3-Naphthalenes
C4-Naphthalenes
Acenaphthene
Acenaphthylene
Biphenyl
Fluorene
C1-Fluorenes
C2-Fluorenes
C3-Fluorenes
Dibenzofuran
Dibenzothiophene
C1-Dibenzothiophenes
C2-Dibenzothiophenes
C3-Dibenzothiophenes
C4-Dibenzothiophenes
Phenanthrene
Anthracene
C1-Phenanthrenes/Anthracenes
C2-Phenanthrenes/Anthracenes
C3-Phenanthrenes/Anthracenes
C4-Phenanthrenes/Anthracenes
CP1
CP2
CP3
CP4
CP5
CP6
A

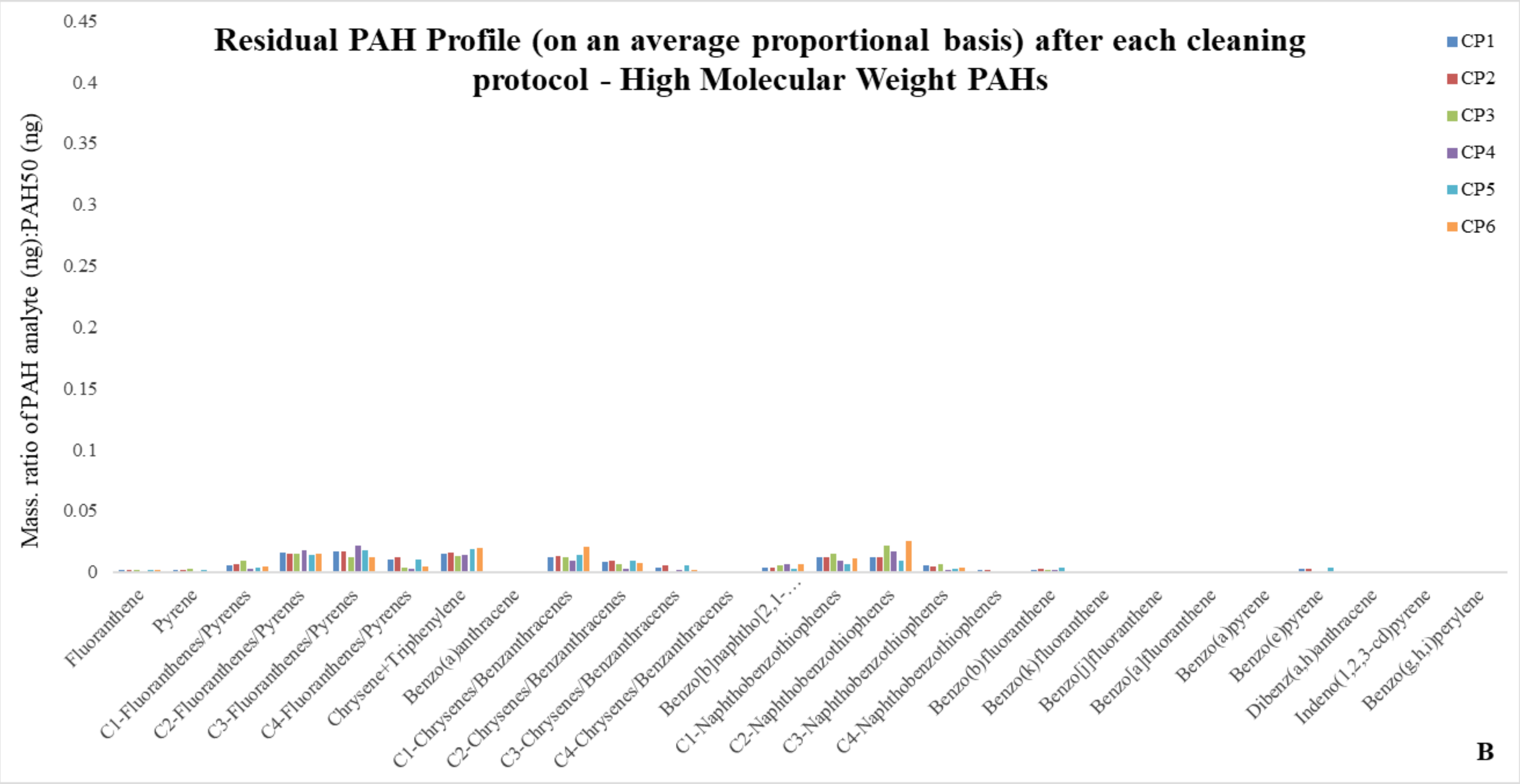


Figure 5A-B: The average proportional residual PAH profile after each cleaning protocol. For ease for viewing, the PAH50 profile was separated into low molecular weight PAHs (A) and high molecular weight PAHs (B). Low molecular weight PAHs contain three or fewer rings; high molecular weight PAHs have four or more rings. Some light weight PAHs are observed (i.e. naphthalene, C2-naphthalenes), however, looking at the sediment profiles for Slick B (Figure 1), these PAHs are not present. It is thought that these PAHs are related laboratory process contamination rather than the cleaning process. Error bars are one standard deviation.

Conclusions

Six field cleaning protocols were tested with two different spiked sediment types. Even at these worst-case sediment scenarios, the highest residual percentages of TEH and PAH50 in any of the cleaning protocol replicates were 0.032% and 0.029% respectively. Generally, the cleaning protocols left hydrocarbon residues that were ≤ 0.01% of the amount in the sediment sample. This indicates that regardless of decontamination protocol or sediment type, the TEH and PAH50 residue remaining is so minimal that it would be indistinguishable from other sources of field sampling or laboratory analytical variability. While statistically significant differences were detected in the relative efficacies of different cleaning protocols (i.e., protocols that included a solvent step removed more hydrocarbons from the equipment than those that did not), the differences were inconsequential given that only very low hydrocarbon residues were measured in all cases.

From these results, it was observed that cleaning protocols that used solvent as part of the process were more effective than protocols that did not. Unfortunately, bringing solvent bottles into the field is not always a feasible option and the use of isopropyl alcohol wipes represents an effective and practical alternative to solvents such as acetone and hexane. Ultimately, although the results of this study of oil-spiked sediments indicated that cross-contamination from properly cleaned and reused field sampling equipment was negligible, good field sampling practices should continue to be followed, including the practice of collecting equipment blanks as ordained by the sampling plan, and, if possible, the sequencing of sample collection from areas of least to areas of most expected contamination.

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Supplemental Information

$$LOD_{MDPA_{ji}} = \frac{ISm_{ji} \times \left(\frac{SAC_i}{ISa_{ji}} - b\right)}{m}$$

Where:

j = *sample*

i = *analyte*

ISa = *internal standard area*

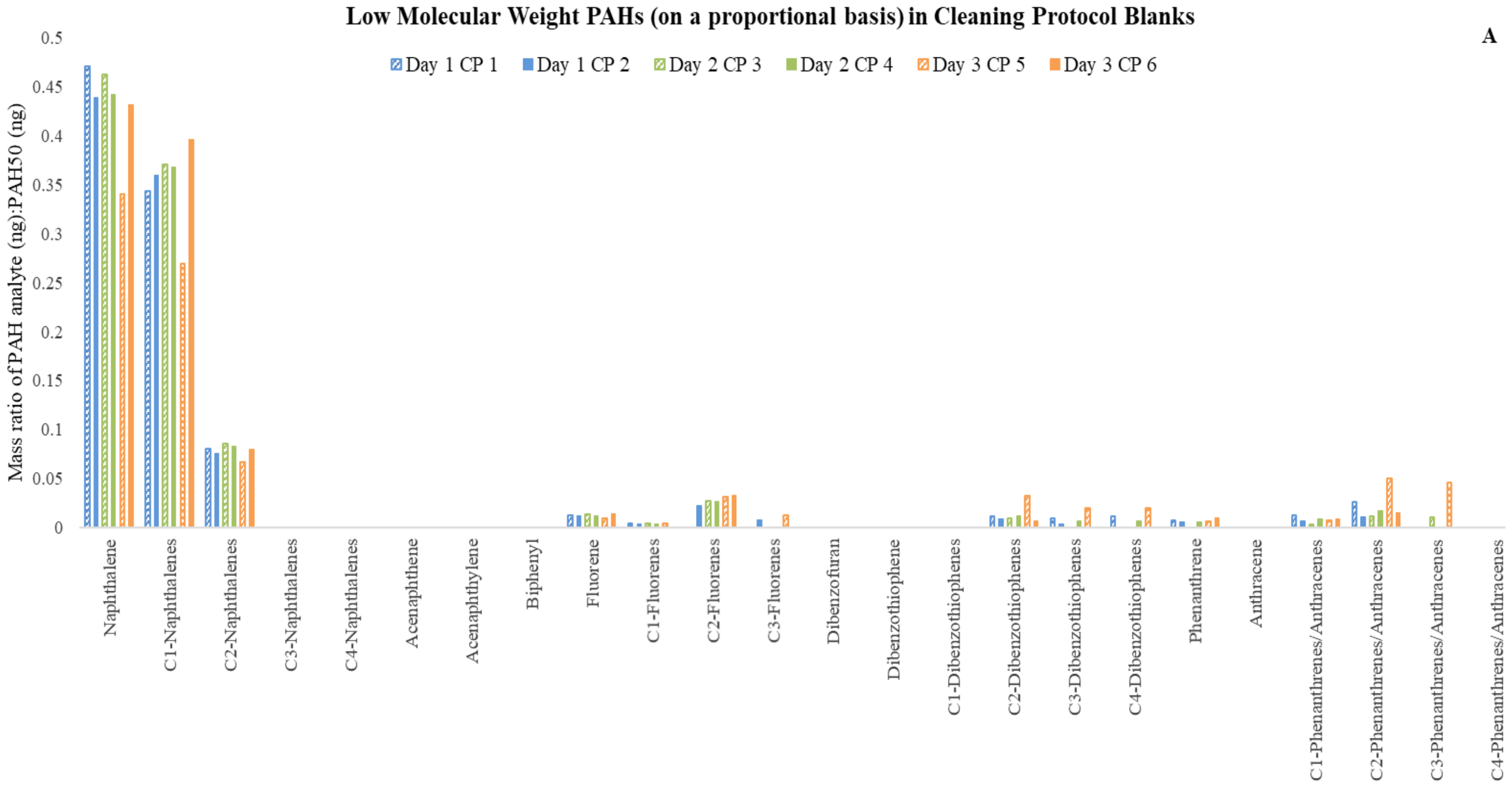
ISm = *internal standard mass*

SAC_i = *average* $MDPA_i + 3\sigma_{MDPAi}$

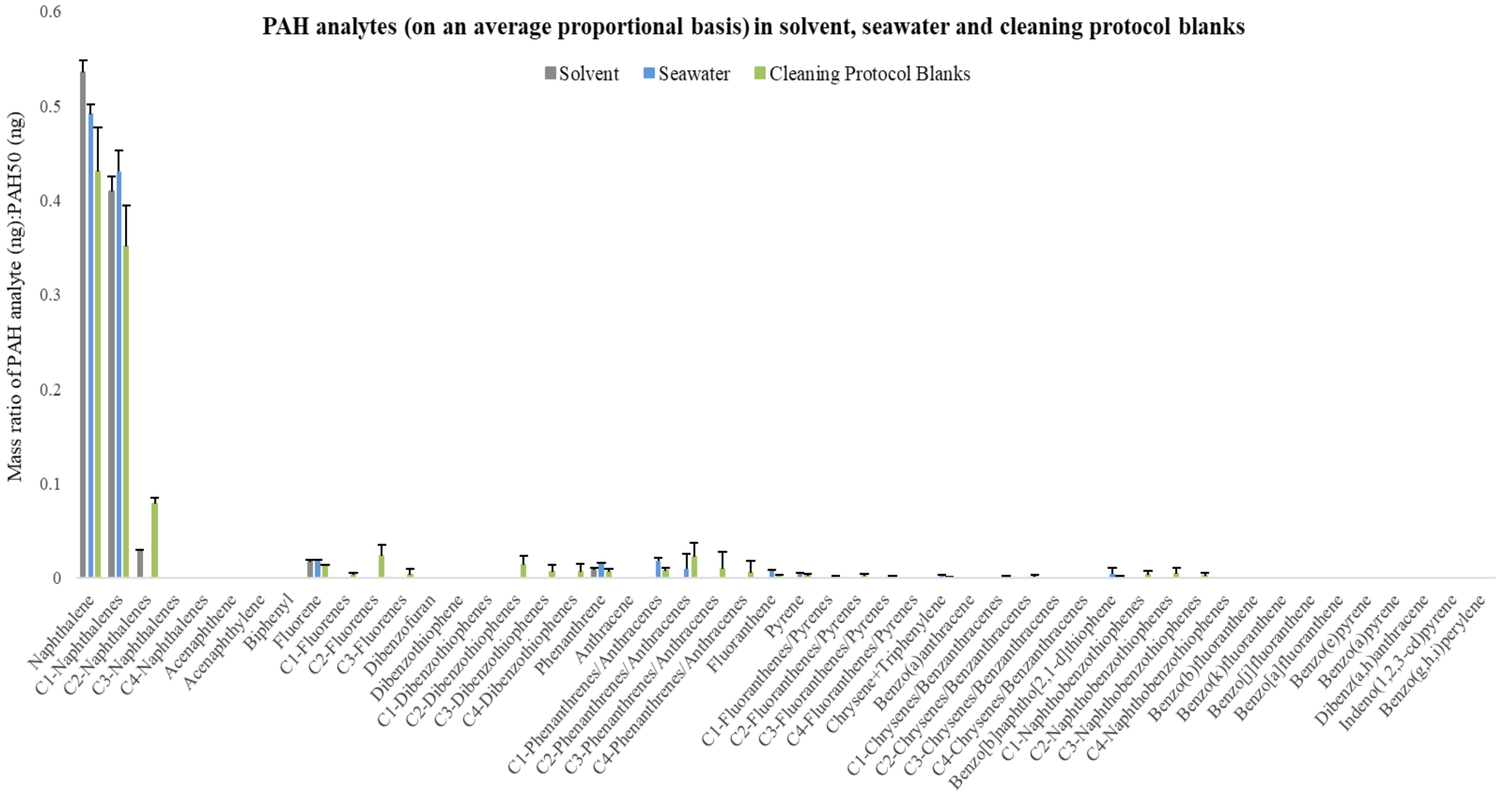
m = *calibration slope*

b = *calibration intercept*

Supplemental Figure 1: Equation for calculating individual PAH MDLs.


Low Molecular Weight PAHs (on a proportional basis) in Cleaning Protocol Blanks
A
▨ Day 1 CP 1
■ Day 1 CP 2
▨ Day 2 CP 3
■ Day 2 CP 4
▨ Day 3 CP 5
■ Day 3 CP 6
Mass ratio of PAH analyte (ng):PAH50 (ng)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Naphthalene
C1-Naphthalenes
C2-Naphthalenes
C3-Naphthalenes
C4-Naphthalenes
Acenaphthene
Acenaphthylene
Biphenyl
Fluorene
C1-Fluorenes
C2-Fluorenes
C3-Fluorenes
Dibenzofuran
Dibenzothiophene
C1-Dibenzothiophenes
C2-Dibenzothiophenes
C3-Dibenzothiophenes
C4-Dibenzothiophenes
Phenanthrene
Anthracene
C1-Phenanthrenes/Anthracenes
C2-Phenanthrenes/Anthracenes
C3-Phenanthrenes/Anthracenes
C4-Phenanthrenes/Anthracenes

Supplemental Figure 2: PAH analyte proportions for each cleaning protocol blank. For ease for viewing, the PAH50 profile was separated into low molecular weight PAHs (A) and high molecular weight PAHs (B). Low molecular weight PAHs contain three or fewer rings; high molecular weight PAHs have four or more rings.



Supplemental Figure 3: PAH 50 profile for PAHs measured in the solvent, seawater and cleaning protocol blanks (the blanks from each of the six protocols were averaged together). The error bars are standard deviation.

Supplemental Table 1: Grain-size analysis and Particle (Φ) Distribution

		Folly River		Leadenwah Creek	
	Φ Fraction	Percent (%) Composition	% Sediment Fraction	Percent (%) Composition	% Sediment Fraction
Gravel	-1	0	0	0	0
Sand	0	0	93.8	0	48.8
	1	0.242		0.846	
	1.5	0.107		0.172	
	2	1.39		1.81	
	2.5	7.56		7.12	
	3	50.9		24.0	
	3.5	31.2		11.8	
	4	2.43		3.02	
Silt	4	0.186	6.2	0.484	51.2
	4.5	0.371		4.25	
	5	0.047		0.147	
	5.5	0.191		0.125	
	6	0.174		1.79	
	6.5	0.238		2.09	
	7	0.514		2.21	
	7.5	0.279		14.6	
Clay	8	0.346		10.8	
	8.5	0.149		1.23	
	9	3.70		13.8	