CytoCulture
ENVIRONMENTAL
BIOTECHNOLOGY
A Division Of CytoCulture International Inc.

December 14, 1995

Client: **Subsurface Consultants, Inc.**
171 12th Street
Suite 201
Oakland, CA 94607

FAX (510) 268-0137

Contact: Jeri Alexander

Client Code: 447-055 **Project Description:** Connell Olds

SAMPLES: Four groundwater samples were received on 12/04/95 in 1 liter glass sample bottles. The samples were stored at 4°C and assayed within 24 hours.

Hydrocarbon-Degrading Bacteria Plate Enumeration Assay Results

ANALYSIS REQUEST: Bacterial enumeration for petroleum hydrocarbon-degraders (target hydrocarbons: Gasoline / Diesel and Motor Oil)

CARBON SOURCE: Diesel (Chevron #2) and motor oil were blended in a 50:50 ratio as the sole carbon and energy sources for the growth of hydrocarbon-degrading aerobic bacteria on agar plates. Gasoline (Chevron Reg.) was added to the lids to provide petroleum hydrocarbon vapors.

PROTOCOL: Sterile agar plates (100 x 15 mm) were prepared with minimal salts medium at pH 6.8 with 1.5% noble agar, without any other carbon sources or nutrients added. A 200 µl aliquot of pasteurized gasoline was added to absorbent paper in the plate lids to provide a vapor source of hydrocarbons on 12/05, 12/06 and 12/08. Each plate was inoculated with 100 µl of each water sample, or a log dilution of each sample. Triplicate plates were inoculated at sample dilutions of 10^0, 10^{-1} and 10^{-2}. The plates were spread on 12/05/95. The plates were counted after 9 days in a humidified incubator at 30°C on 12/14/95* The plate count data are reported as colony forming units (**cfu**) per milliliter (ml) of sample as an average of the plate count data obtained with inoculations at the 3 dilutions.

* Due to power outage, the normal 5-7 days incubation period was extended to 9 days. However, inspection of the plates at 4 days vs. 9 days indicated no significant differences in colony densities (colonies simply grew larger, not more numerous).

SAMPLE NUMBER	SAMPLE DATE	COUNT DATE	HYDROCARBON-DEGRADERS CFU PER MILLILITER
MW-1	12/04/95	12/14/95	$< 1.5 \times 10^2$
MW-8	12/04/95	12/14/95	1.6×10^2
MW-9	12/04/95	12/14/95	6.5×10^2
MW-10	12/04/95	12/14/95	$< 1.5 \times 10^2$

NUTRIENT ASSAYS and pH RESULTS

ANALYSIS REQUEST: Nutrient assays for ammonia, nitrate and ortho-phosphate levels. pH measurements on fresh samples. Dissolved oxygen data was collected by the client at the site.

PROTOCOL: Colorimetric assays were performed for the determination of ammonia, nitrate and ortho-phosphate levels in water samples, per EPA manual colorimetric protocols using Hach reagents and a Gilford 340 VIS/UV digital spectrophotometer. The pH was measured with a Corning digital pH meter. Mean pH values were obtained from triplicate measurements. All assays conform to California CLP and Standard Water & Wastewater analytical method specifications.

SAMPLE NUMBER	SAMPLE DATE	Ammonia (mg/L) N	Nitrate (mg/L) N	Phosphate (mg/L) P	pH
MW-1	12/04/95	6.5	0.12	0.50	6.7
MW-8	12/04/95	0.20	0.39	0.50	6.5
MW-9	12/04/95	0.65	< 0.01	0.35	6.2
MW-10	12/04/95	1.8	0.11	0.25	6.6

COMMENTS:

Generally, the groundwater samples appeared to have low (background) levels of ammonia, nitrate and phosphate nutrients, with the possible exception of monitoring well MW-1 sample. Ammonia nitrogen level was elevated in the MW-1 sample, although there was also a slight turbidity in this sample, even after filtration through Whatman No. 1 filer paper. This turbidity could have caused an elevated absorbance signal in the colorimetric assay. MW-1 also had a strong petroleum (gasoline) odor, and evidence of trace free product floating on the sample. MW-10 sample also had a petroleum odor of lesser intensity. MW-1 and MW-10 nonetheless had low to non-detectable populations of gasoline degrading bacteria, whereas MW-8 and MW-9 had slightly higher populations. pH levels in all samples were typical or slightly acidic. Diminished oxygen and nutrient levels presumably contributed to the low bacterial populations.

Please contact us at 510-233-0102 to discuss these data and interpretation of the results.

Thank you.

Assay performed by:

Sue Arve, M.S.
Director of Technical Services

Approved by:

Randall von Wedel, Ph.D.
Director of Research

b:\ssc-con.95

SSC-CON

CytoCulture

ENVIRONMENTAL BIOTECHNOLOGY

A DIVISION OF CYTOCULTURE INTERNATIONAL INC.

Chain of Custody Form

Project Description: Connell Olds Code/P.O. 447.055

Client: Subsurface Consultants Client Contact: Jeri Alexander

Address to send results: 171 12th St., Suite 201 Oakland, Ca.

Tel.: (510) 268.0461 Fax: (510) 268-0137 Zip: 94607

Sampler: Dennis Alexander Recorder:

Matrix		Sample Number	Sampling		Sample Notes	Analysis					
S	W		Date	Time		CFU	pH	DO	NH_3	PO_4	NO_3
	X	MW-1	12/4/95	0900	Dissolved oxygen 1.3 ppm	X	X		X	X	X
	X	MW-9	12/4/95	1215	Dissolved oxygen 2.2 ppm	X	X		X	X	X
	X	MW-8	12/4/95	1030	DO = 2.7 ppm	X	X		X	X	X
	X	MW-10	12/4/95	1300	DO = 1.3 ppm	X	X		X	X	X

Laboratory Notes:

Use diesel: motor oil / gasoline plates w/ 1.5% Agar.
Replenish gasoline in lids at 24, 48 hr.

Predominant Hydrocarbon Contaminants: GAS

Chain of Custody Record

Relinquished by:	Date/Hr.	Received by:	Date/Hr.
Dennis Alexander	12/4/95 / 2:55 pm.	[signature]	12-4-95. 2:50 pm
[signature]	12-5-95.	[signature]	12/5/95

cyto/coc11.94

249 TEWKSBURY AVENUE POINT RICHMOND CA 94801
510/233-0102 FAX 510/233-3777

Curtis & Tompkins, Ltd., Analytical Laboratories, Since 1878
2323 Fifth Street, Berkeley, CA 94710. Phone (510) 486-0900

A N A L Y T I C A L R E P O R T

Prepared for:

Subsurface Consultants
171 12th Street
Suite 201
Oakland, CA 94608

Date: 25-SEP-95
Lab Job Number: 122458
Project ID: 447.055
Location: Connell Olds

Reviewed by: _____

Reviewed by: _____

Curtis & Tompkins, Ltd.

LABORATORY NUMBER: 122458
CLIENT: SUBSURFACE CONSULTANTS
PROJECT ID: 447.055
LOCATION: CONNELL OLDS

DATE SAMPLED: 08/30-09/01/95
DATE RECEIVED: 09/01/95
DATE EXTRACTED: 09/05/95
DATE ANALYZED: 09/14/95
DATE REPORTED: 09/25/95
BATCH NO: 23088

Extractable Petroleum Hydrocarbons in Aqueous Solutions
California DOHS Method
LUFT Manual October 1989

LAB ID	CLIENT ID	DIESEL RANGE (ug/L)	REPORTING LIMIT (ug/L)
122458-002	MW-2	150 Y*	50
122458-004	MW-5	180 Y*	50
122458-005	MW-7	170 Y*	50
122458-006	MW-8	240 Y*	50
122458-007	MW-9	680 YL	50
122458-008	MW-10	5,900 YL	50
122458-009	MW-11	240 Y*	50
METHOD BLANK	N/A	ND	50

ND = Not detected at or above reporting limit.

* Possible laboratory contamination.
Y = Sample chromatogram does not resemble Diesel standard.
L = Lighter hydrocarbons contributing to quantitated area.

QA/QC SUMMARY: BS/BSD

RPD, %	<1
RECOVERY, %	106

Curtis & Tompkins, Ltd.

LABORATORY NUMBER: 122458
CLIENT: SUBSURFACE CONSULTANTS
PROJECT ID: 447.055
LOCATION: CONNELL OLDS

DATE SAMPLED: 08/31/95
DATE RECEIVED: 09/01/95
DATE EXTRACTED: 09/14/95
DATE ANALYZED: 09/18/95
DATE REPORTED: 09/25/95
BATCH NO: 23266

Extractable Petroleum Hydrocarbons in Aqueous Solutions
California DOHS Method
LUFT Manual October 1989

LAB ID	CLIENT ID	DIESEL RANGE (ug/L)	REPORTING LIMIT (ug/L)
122458-003	MW-3	ND	50
METHOD BLANK	N/A	ND	50

ND = Not detected at or above reporting limit.

QA/QC SUMMARY: BS/BSD

RPD, %	13
RECOVERY, %	78

Curtis & Tompkins, Ltd.

LABORATORY NUMBER: 122458
CLIENT: SUBSURFACE CONSULTANTS
PROJECT ID: 447.055
LOCATION: CONNELL OLDS

DATE SAMPLED: 08/31/95
DATE RECEIVED: 09/01/95
DATE REQUESTED: 09/08/95
DATE EXTRACTED: 09/11/95
DATE ANALYZED: 09/22/95
DATE REPORTED: 09/25/95
BATCH NO: 23197

Extractable Petroleum Hydrocarbons in Aqueous Solutions
California DOHS Method
LUFT Manual October 1989

LAB ID	CLIENT ID	DIESEL RANGE (ug/L)	REPORTING LIMIT (ug/L)
122458-010	MW-13	ND	50
METHOD BLANK	N/A	ND	50

ND = Not detected at or above reporting limit.

QA/QC SUMMARY: BS/BSD

RPD, %	2
RECOVERY, %	95

CHAIN OF CUSTODY FORM

122458

PAGE ____ OF ____

PROJECT NAME: Connell Olds
JOB NUMBER: 447.055
PROJECT CONTACT: Jeri Alexander
SAMPLED BY: Dennis Alexander
LAB: Cutlist-Tompkins
TURNAROUND: Normal
REQUESTED BY: Jeri Alexander

LABORATORY I.D. NUMBER	SCI SAMPLE NUMBER	MATRIX: WATER	SOIL	WASTE	AIR	CONTAINERS: VOA	LITER	PINT	TUBE	METHOD PRESERVED: HCL	H_2SO_4	HNO_3	ICE	NONE	SAMPLING DATE: MONTH	DAY	YEAR	TIME	NOTES	ANALYSIS REQUESTED: TEH	TVH/BTXE	DCA	O+G	Semi-Volatiles
-1	MW-1	X					2			X			X		08	30	95	1230					X	X
-2	MW-2	X				4	1			X			X		08	30	95	1445		X	X	X		
-3	MW-3	X				4	1			X			X		08	31	95	1000		X	X	X		
-4	MW-5	X				4	1			X			X		08	31	95	0915		X	X	X		
-5	MW-7	X				4	1			X			X		08	31	95	0830		X	X	X		
-6	MW-8	X				4	1			X			X		09	01	95	1045		X	X	X		
-7	MW-9	X				4	1			X			X		08	31	95	1130		X	X	X		
-8	MW-10	X				4	1			X			X		09	01	95	1215		X	X	X		
-9	MW-11	X				4	1			X			X		08	30	95	1330		X	X	X		
-10	MW-13	X				4	1			X			X		08	31	95	0800		X	X	X		

CHAIN OF CUSTODY RECORD

RELEASED BY: (Signature)	DATE / TIME	RECEIVED BY: (Signature)	DATE / TIME
Dennis Alexander	9/1/95 2:30 p.m.	Damara Mone	9/1/95 2:25
RELEASED BY: (Signature)	DATE / TIME	RECEIVED BY: (Signature)	DATE / TIME
RELEASED BY: (Signature)	DATE / TIME	RECEIVED BY: (Signature)	DATE / TIME
RELEASED BY: (Signature)	DATE / TIME	RECEIVED BY: (Signature)	DATE / TIME

COMMENTS & NOTES:

Subsurface Consultants, Inc.
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