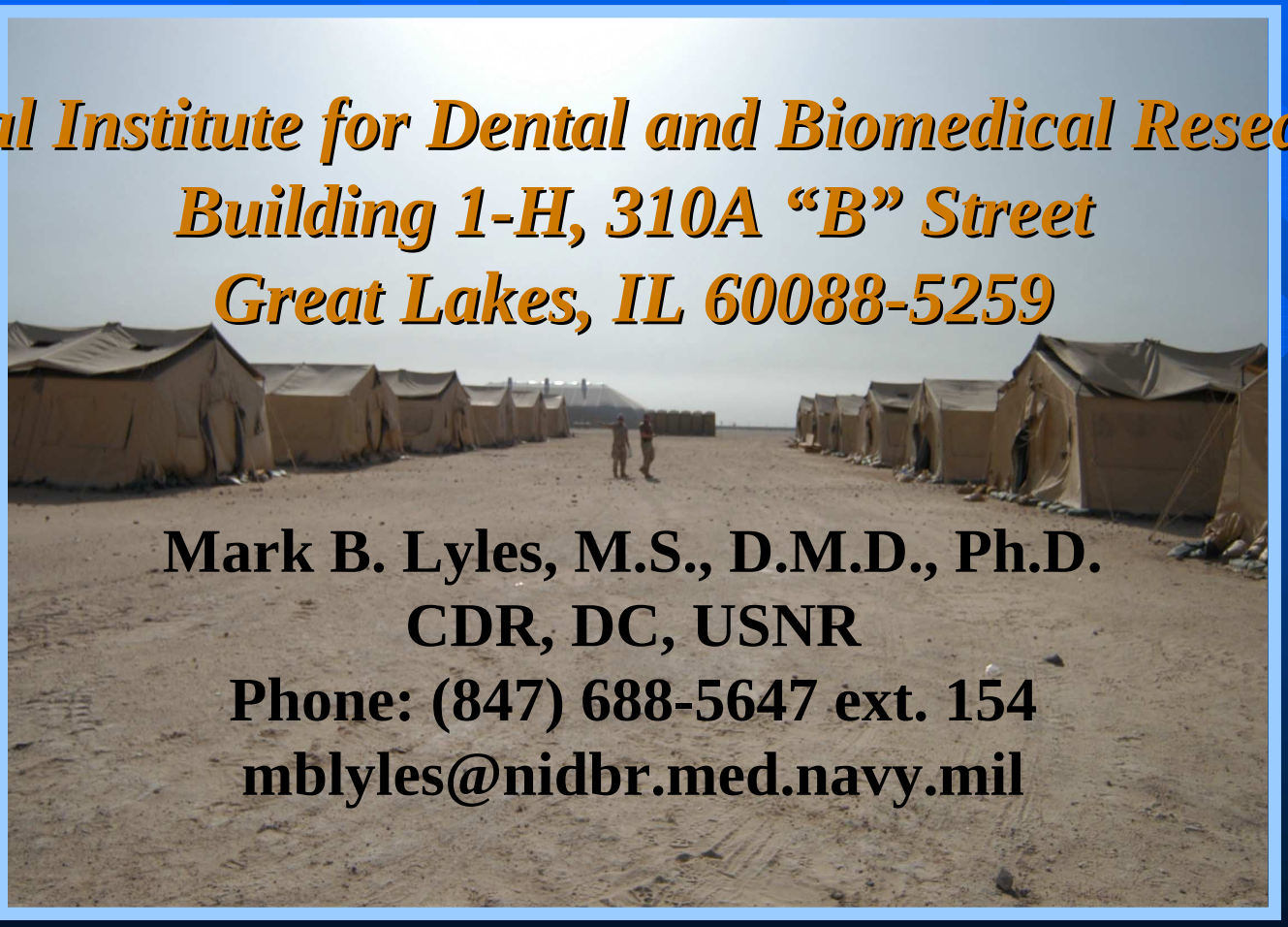
The Physical, Biological and Chemical Characterization of Micro-particulates from the Middle East



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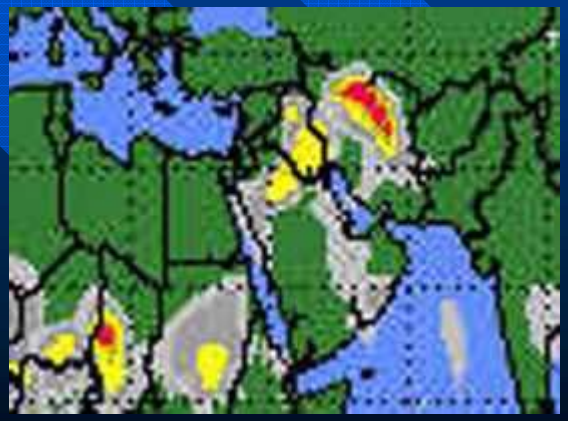
Heavy
Dust
Dust-Free
Area
Dust
Iraq
B
Dust
Saudi
Arabia
A



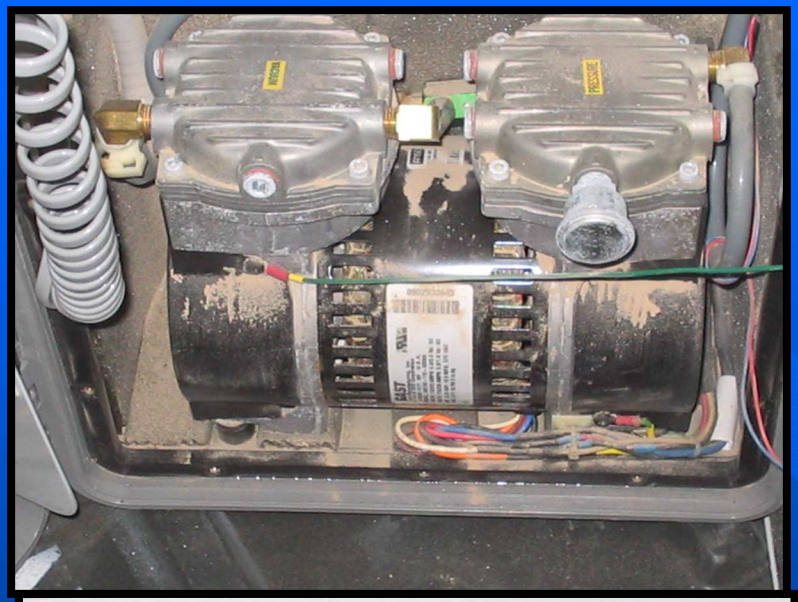
Background

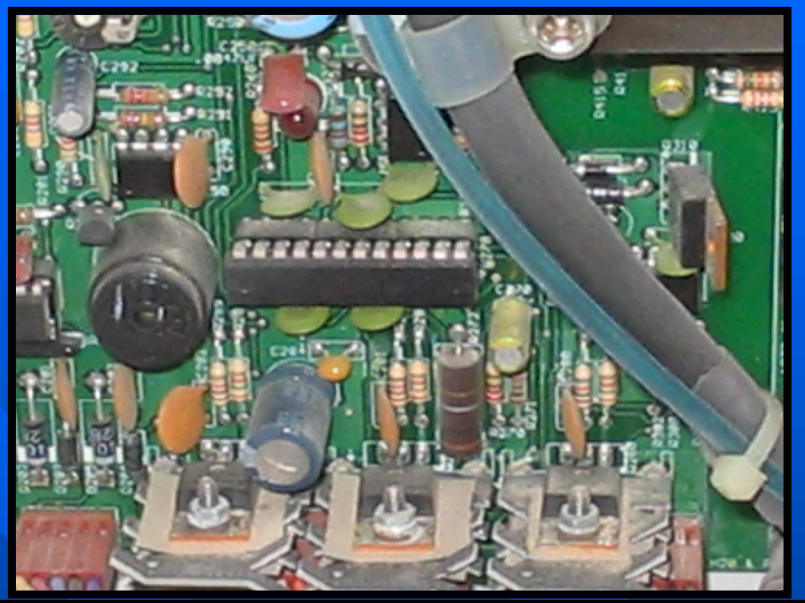
- Dust sample arrives from Iraq October 2003 for ADAL project.
- Scanning Electron Micrograph and Fluorescent Microscopy analysis of dust sample indicates: 1) significant micro-particulate concentration (<20 μm), 2) micro-particulates are not crystalline or are coated, and 3) particles exhibit unique physical properties.
- Pneumonia and other respiratory health problems among US military personnel in the US Central Command Area of Responsibility (USCENTCOM AOR). Significant post-operative infection rate among U.S. and coalition wounded.
- Exposure of airborne micro-particulates, especially during duststorms, is ubiquitous for all coalition forces.
- Information passed to ONR which funds a pilot project.

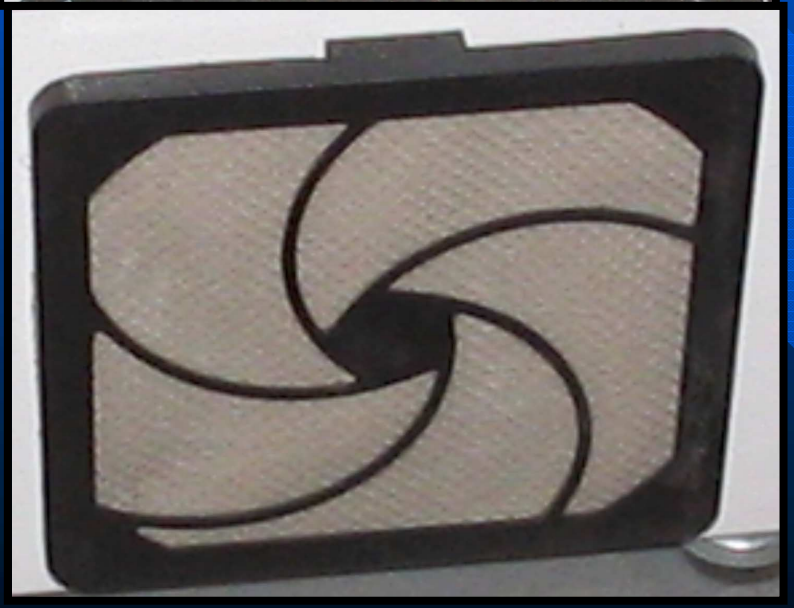
"The Battlespace Environment"
to the 'Warfighter' this means:

- The "terrain", the battlefield *landscape*
- Living *on* the "dirt"
- Traveling *across* the "dirt"
- Fighting *in* the "dirt"
- In Iraq this also means *breathing* the "dirt"

Micro-particulate Exposure



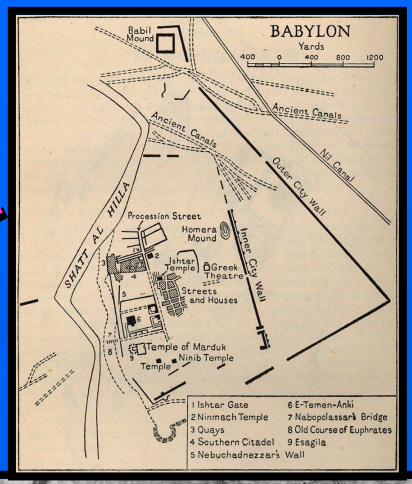




Specific Aims

- ✓ Scientifically collect and process sand/dust samples for transport back to CONUS for further testing.
- ✓ Characterize the sand/dust environment.
- ✓ Physically characterize samples as to particle size distribution and concentration, and other physical qualities.
- ✓ Chemically analyze samples based on size fraction as to elemental content and concentration as well as poly-anion and cation identification.
- ✓ Biologically characterize samples based on size fraction as to anaerobic/ aerobic bacterial populations, yeasts, fungi, and viruses. Special identification of known pathogens.

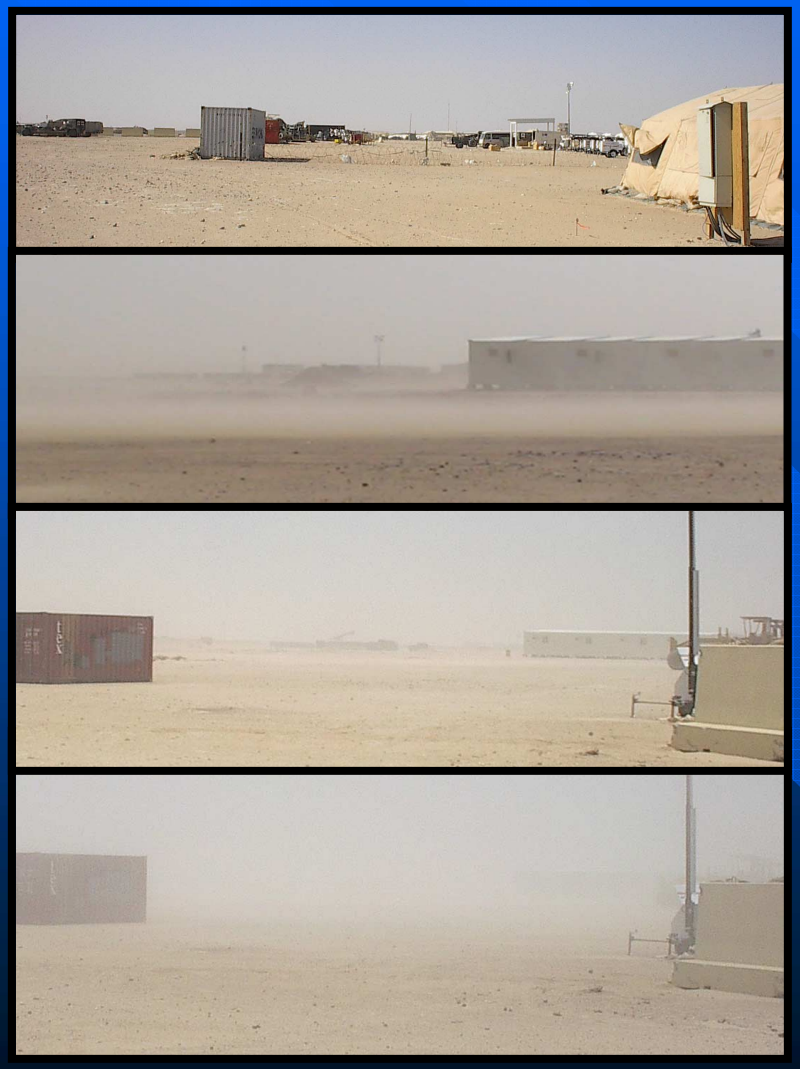
Sampling Locations



Sample Processing

Sieve #	*Pore size (μm)*
40	420
100	149
120	125
230	63
325	44
635	20

Physical Characterization of the Desert Environment



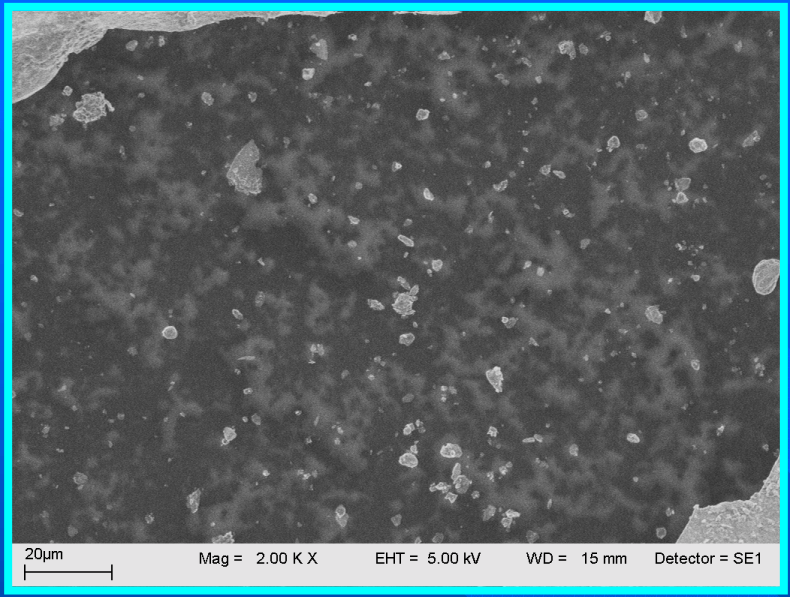
- Particle size distribution & conc.
- Average Wind Velocity
- Humidity
- Temperature

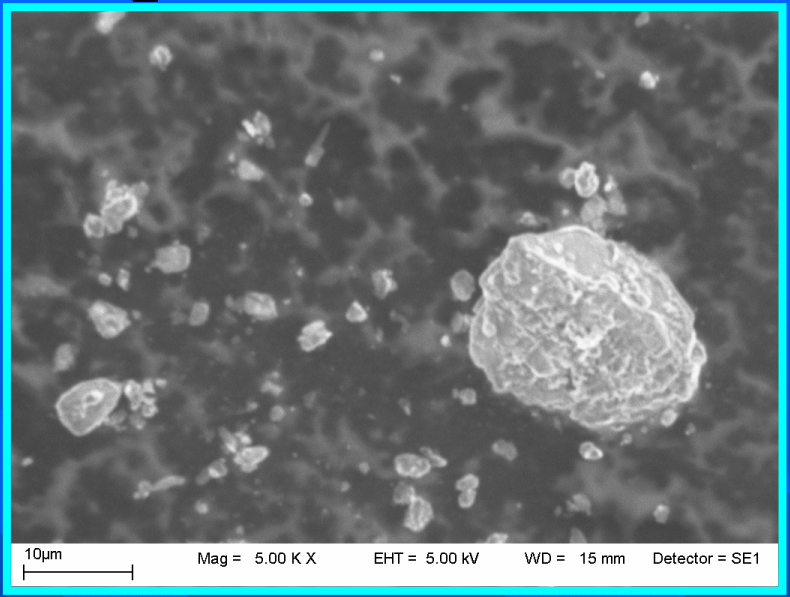
Equipment Used to Characterize the Desert Environment

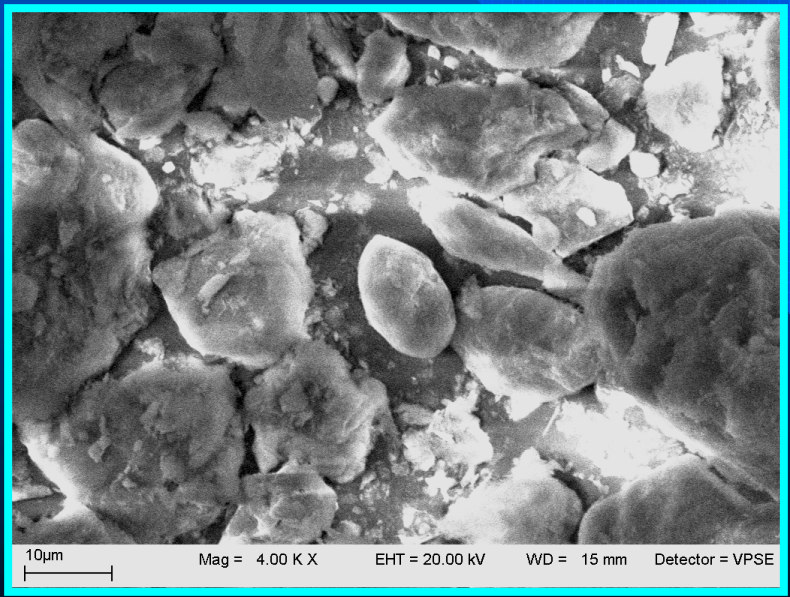
Particle size and distribution, temperature and rel. humidity recorded with a Aerocet 531 hand-held monitoring device (Met One Instruments, Inc.).

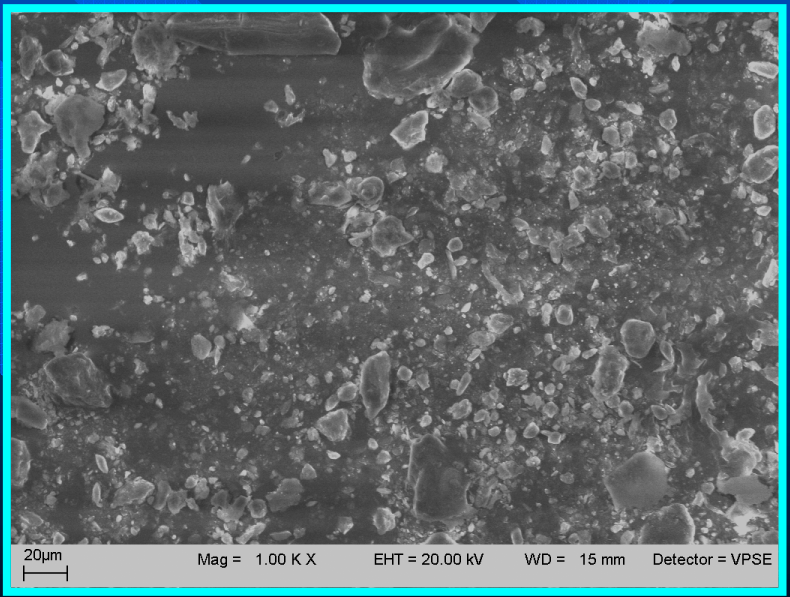
Wind speed monitor (CFM Master Thermo-Anemometer Model DCFM 8901; Mannix Testing and Measurement, Inc.).

Sand / Dust Micro-particulates









Grain Size (um)

700
600
500
400
300
200
100
0

Kuwait
Iraq
Szeged
Ft. Story
Israel
Hawaii
Marshall Is.

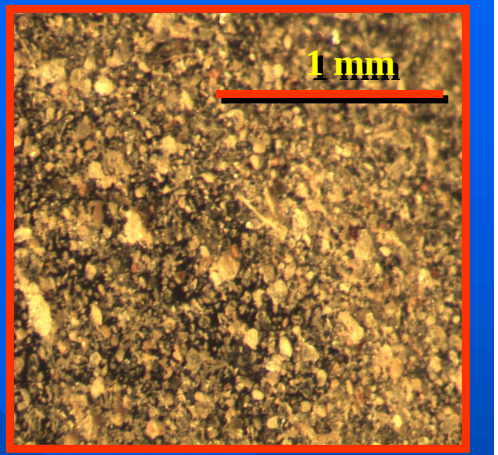
Sand Sites

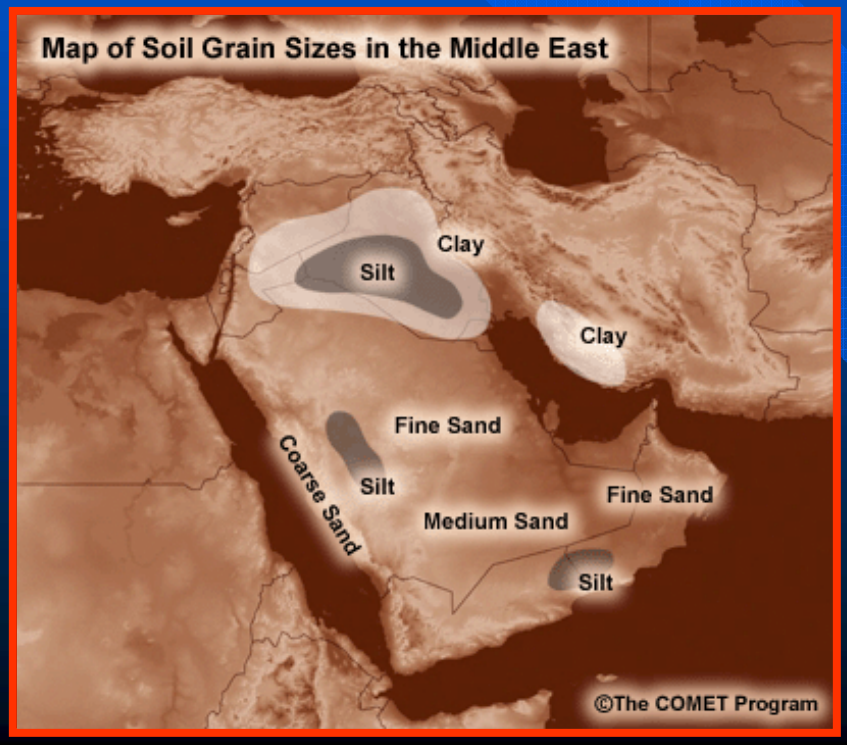
Soils of Lower Mesopotamia

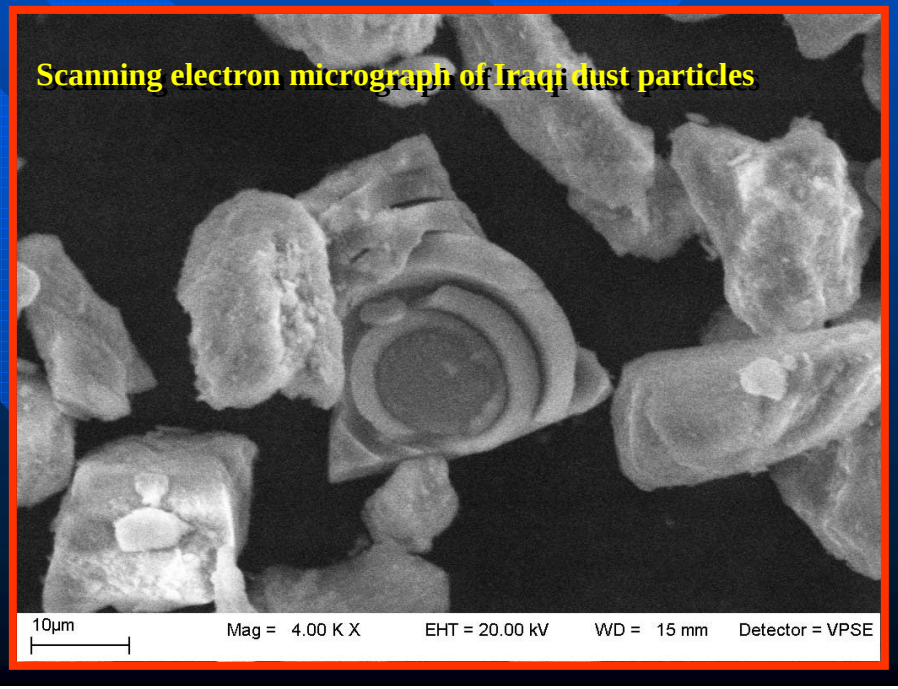
20-60%of surface soil mass is comprised of particles < 2.5 µm in size ($PM_{2.5}$)

As, Cr, Pb, Os are elevated

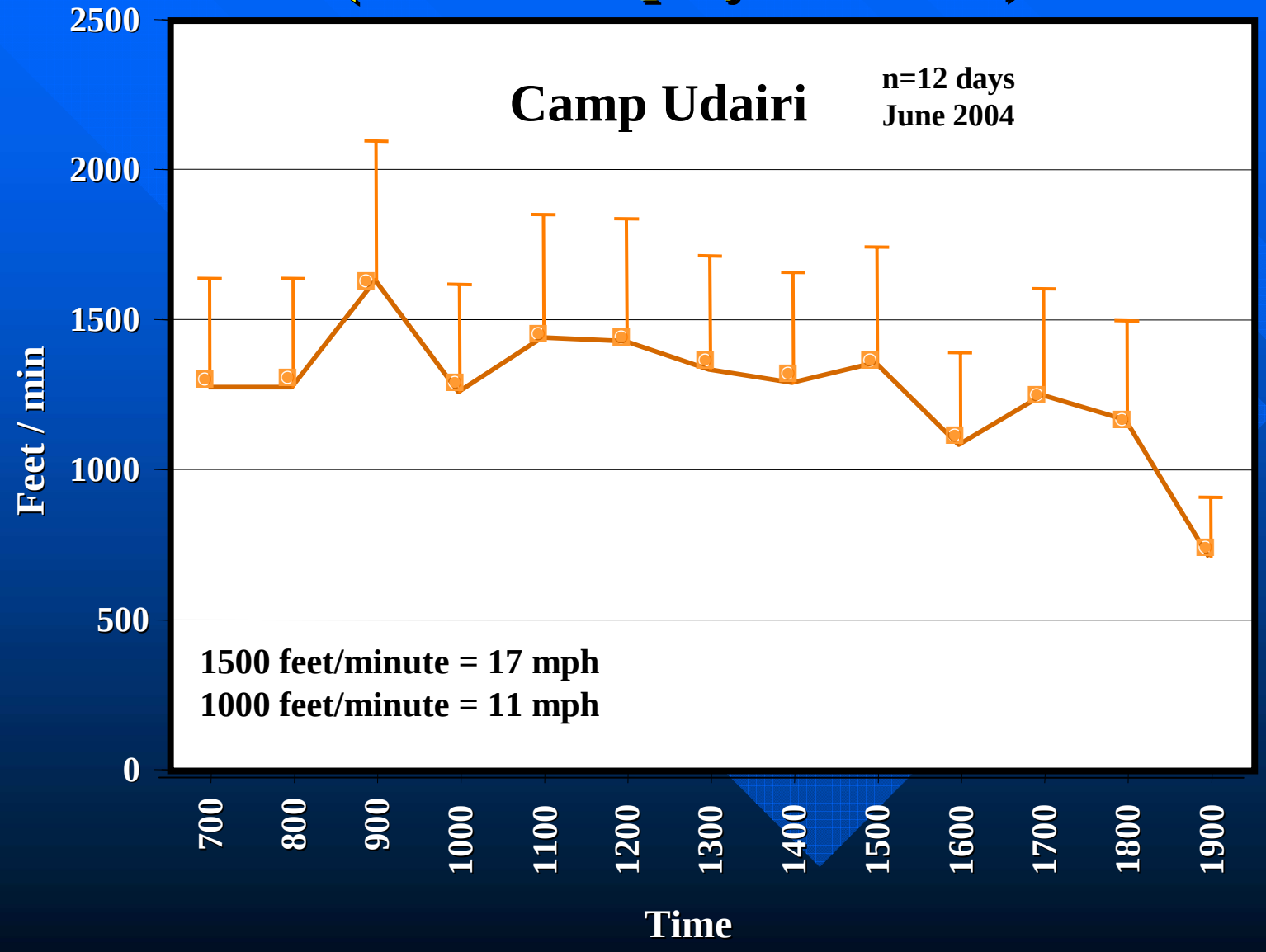
Silicate mineral component mixed with significant proportions of calcium carbonate



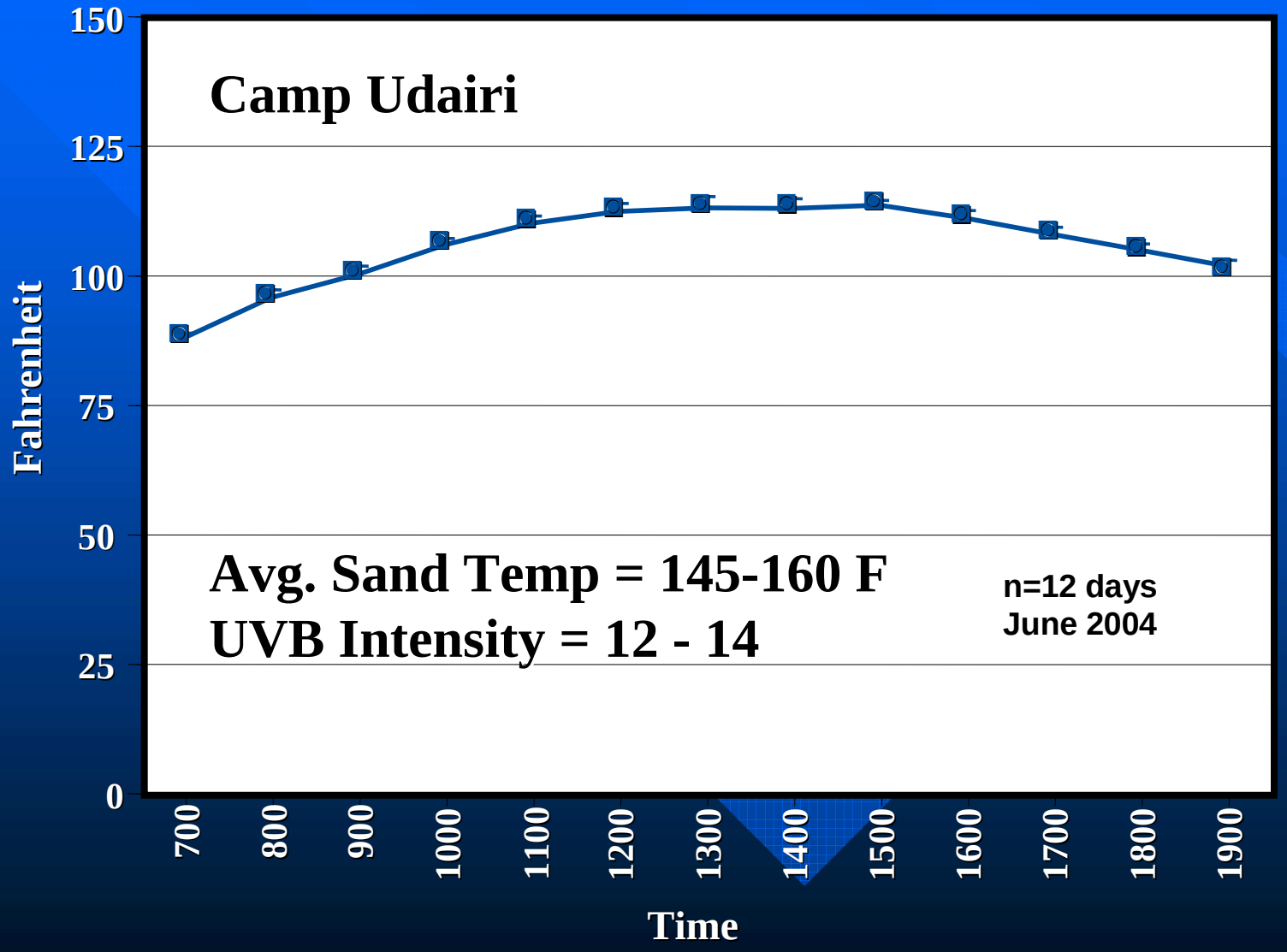




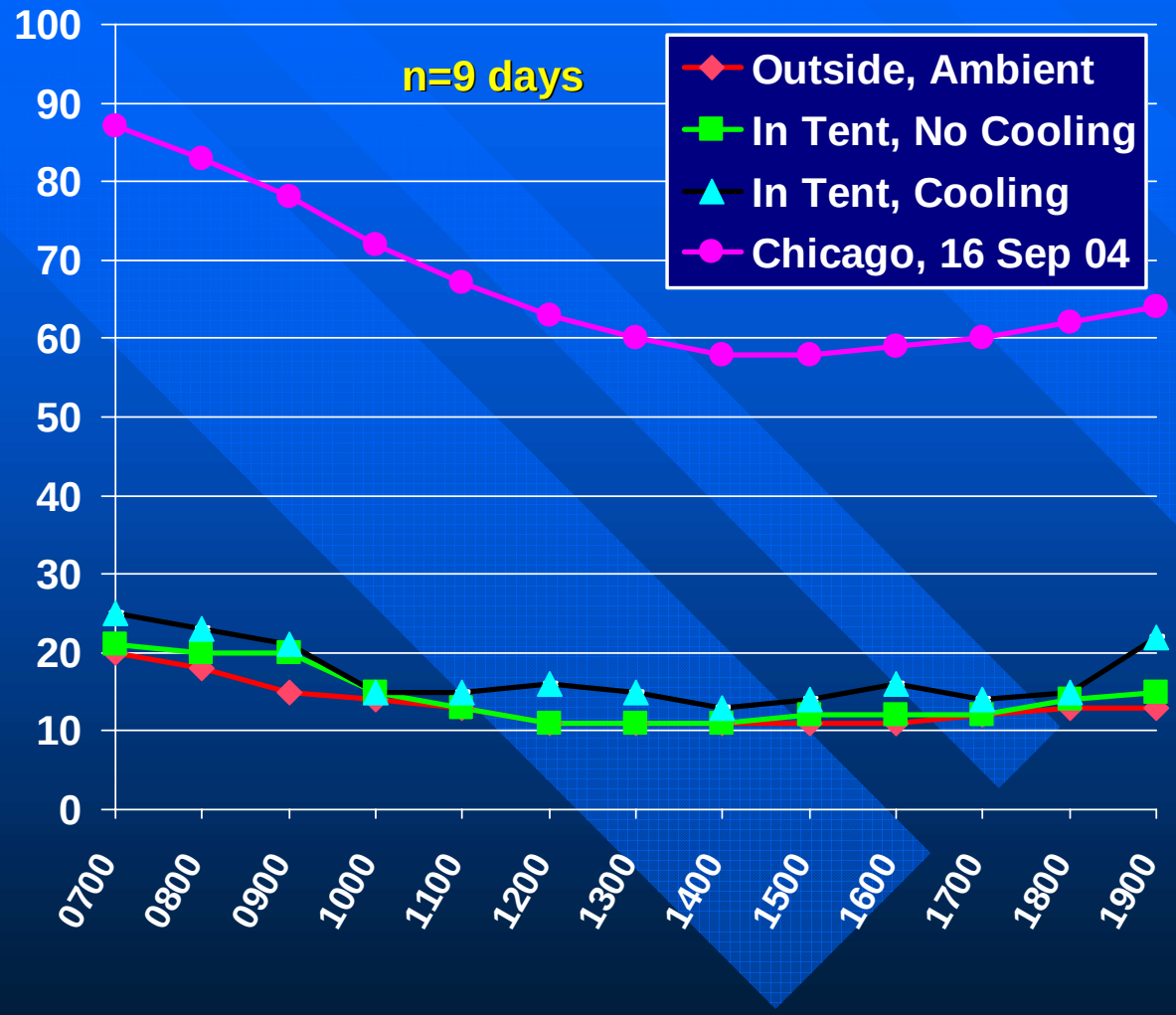
Average Hourly Wind Velocity (Kuwait Deployment Site)



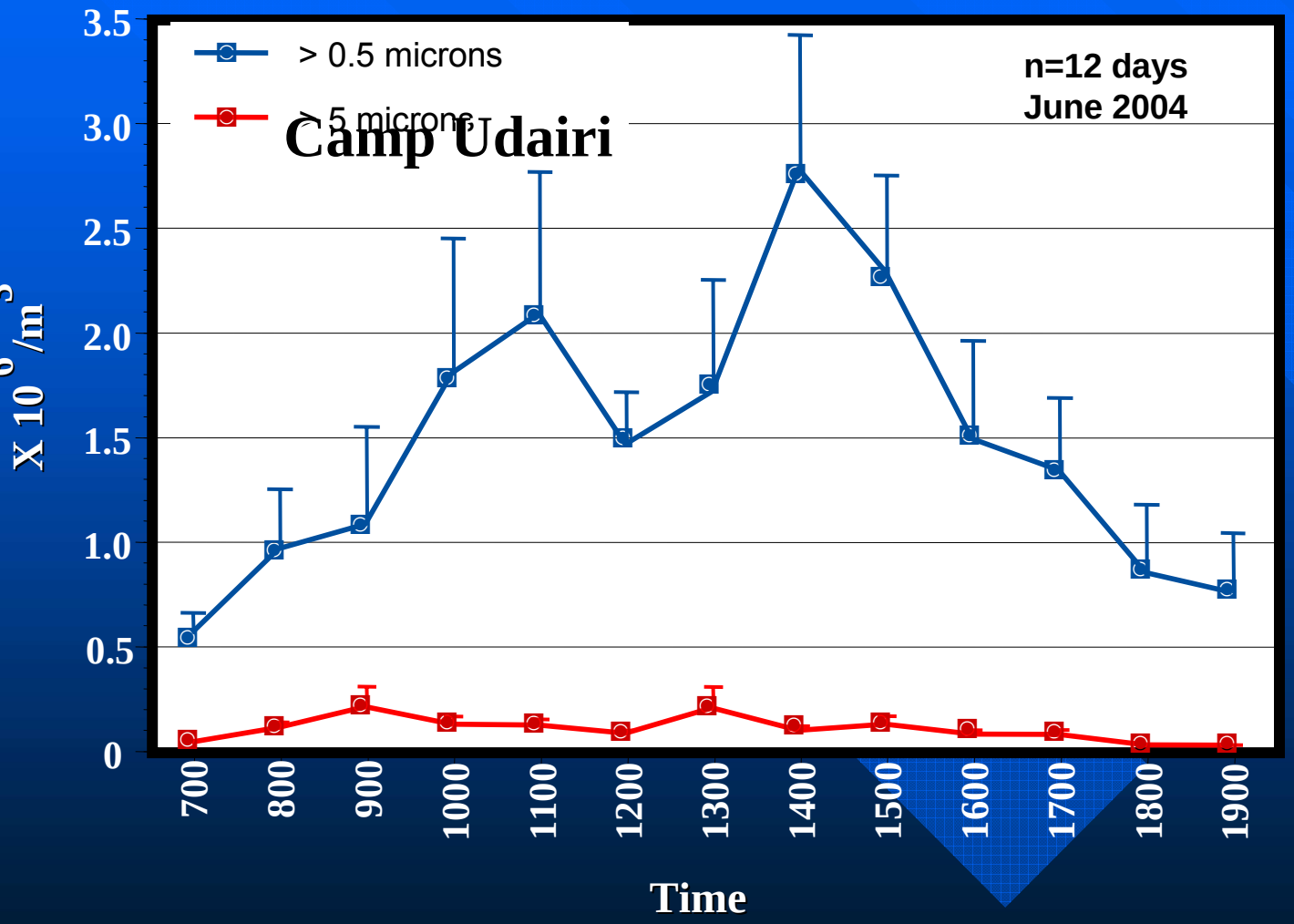
Average Hourly Temperature (Kuwait Deployment Site)



Average Hourly Relative Humidity



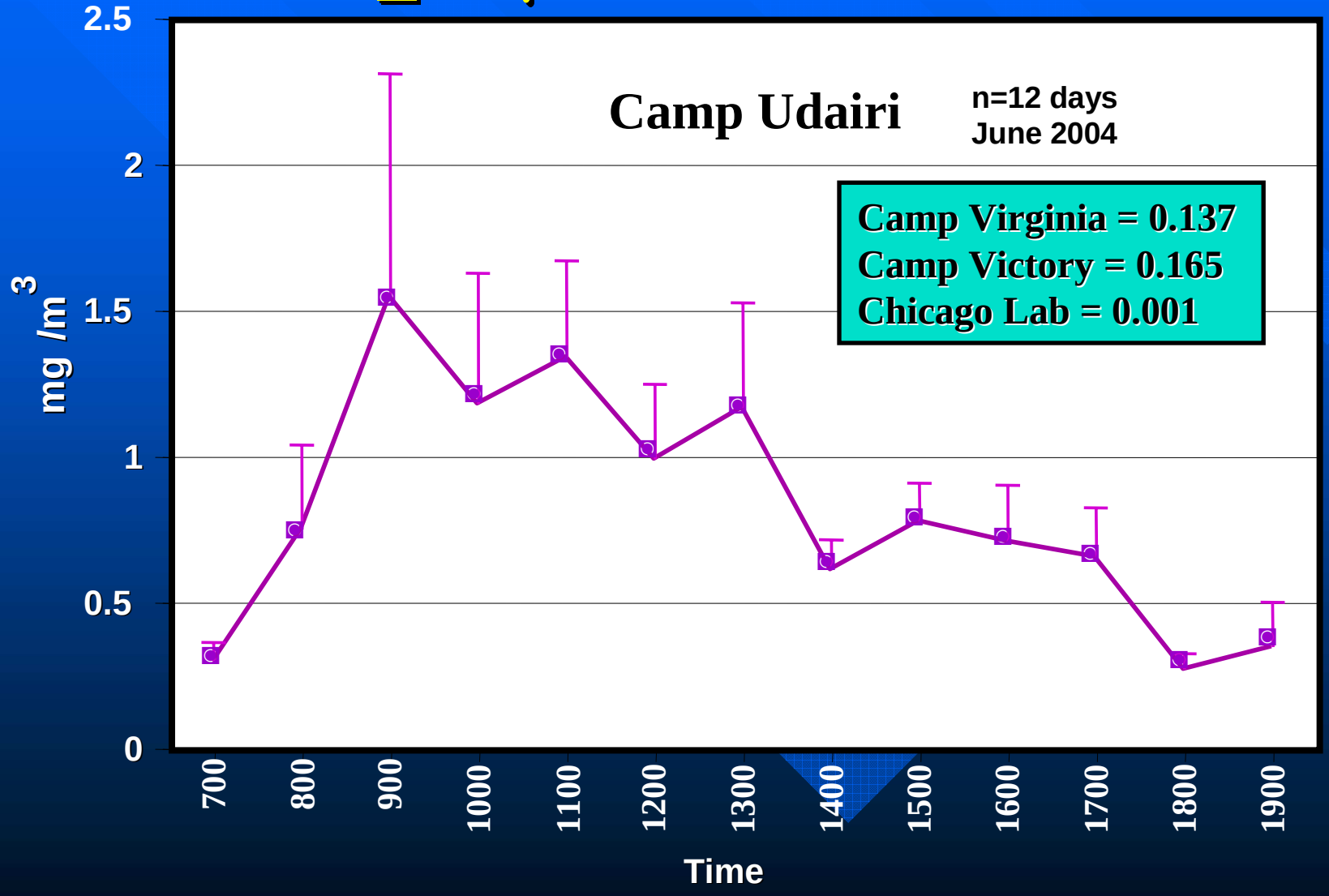
Mean Ambient Airborne Concentrations



>0.5um
Camp Virginia = 348,040
Camp Victory = 201,510
Chicago Lab = 37,234

>5um
Camp Virginia = 14,380
Camp Victory = 13,150
Chicago Lab = 1,034

Mean Total Suspended Particle Mass ≤ 10 μm in Ambient Air



Chemical Analysis: Heavy Metals

Acid Extractables
Tent 1

Particles less than 250 µm are airborne at 15 MPH.

20-40 µm deposited in nose, throat, trachea.

20-10 µm deposited in all upper airways of the lung.

≤10 µm deposited in lowest airways of lungs.

98% of Tent Dust is less than 149 um.

~ 60% of Sand Samples are less than 149 um.

Sample	>120um	>90um	> 63um	>44um	>20um	<20um
Mass	0.2627	0.2596	0.2488	0.2626	0.2441	0.2504
Element	% dry wt	%dry wt	%dry wt	%dry wt	%dry wt	%dry wt
Sr	0.0697	0.0642	0.0995	0.1978	**0.2718**	0.2436
Ba	0.0068	0.0072	0.0081	0.0192	0.0308	**0.0463**
P	0.0160	0.0170	0.0234	0.0433	0.0549	**0.0649**
S	2.4413	2.4230	3.0444	**4.0062**	3.6646	3.0458
Mg	0.6844	0.8718	1.2672	1.5505	1.7234	**1.7784**
V	0.0022	0.0026	0.0032	0.0041	0.0046	**0.0049**
Na	0.1759	0.1963	0.1672	0.2056	0.2123	**0.2225**
Al	0.2969	0.3832	0.4948	0.6351	0.7164	**0.7521**
Ca	9.0134	10.3057	11.7495	13.9148	15.3535	**16.7133**
Zn	0.0053	0.0039	0.0042	0.0070	0.0112	**0.0206**
Cu	0.0060	0.0050	0.0036	0.0054	0.0077	**0.0268**
Ni	0.0089	0.0094	0.0169	0.0197	0.0305	**0.0564**
Y	0.0009	0.0006	0.0006	0.0007	0.0009	**0.0010**
K	0.0502	0.0653	0.0612	0.0942	0.1186	**0.1422**
Mn	0.0174	0.0222	0.0268	0.0305	0.0331	**0.0352**
Fe	0.3506	0.4844	0.6889	0.8419	0.9601	**0.9736**
Cr	0.0027	0.0032	0.0039	0.0049	0.0052	**0.0052**
Pb	0.0111	0.0038	0.0049	0.0056	0.0076	**0.0138**

Summary

TSP (Total Suspended Particle Mass) (mg/m^3) PM10 (10 µm) and below

= **0.001 mg/m^3** (NIDBR Lab, Great Lakes, IL)
= **0.137 mg/m^3** (Camp Virginia Clinic, Kuwait - indoors)
= **2.469 mg/m^3** (Highest hourly average - 8 AM)
= **9.114 mg/m^3** (Highest TSP reading)
= **2.051 mg/m^3** (Highest daily maximum - 18 June at 1300)
**** NOTE: >9.999 mg/m^3 readings recorded during peak dust storms***

Count (Total Number of Suspended Particles) (/ft^3)

Size Range = 0.5 µm to 10 µm

= **37,234 /ft^3** (NIDBR Lab, Great Lakes, IL)
= **348,040 /ft^3** (Camp Virginia Clinic, Kuwait - indoors)
= **3,037,298 /ft^3** (Highest average hourly maximum at 1300) (SD = 1,556,266/ft^3)
= **16,668,250 /ft^3** (Highest daily maximum – 18 June at 1300)
= **3,614,455 /ft^3** (Highest average daily (0700-1900) maximum 13 June) (SD = 971,589/ft^3)
** NOTE: >20,000,000 counts /ft^3 readings recorded during peak dust storms*

Size Range = 5.0 µm to 10 µm

= **1,034 /ft^3** (NIDBR Lab, Great Lakes, IL)
= **14,380 /ft^3** (Camp Virginia Clinic, Kuwait - indoors)
= **194,945 /ft^3** (Highest average hourly maximum at 1300) (SD = 117,305/ft^3)
= **1,262,120 /ft^3** (Highest daily maximum - 18 June at 1300)
= **148,512 /ft^3** (Highest average daily maximum - 13 June) (SD = 102,861/ft^3)
**** NOTE: >9,999,999 counts/ft^3 readings recorded during peak dust storms***

NOTE: 1 cu ft = 28.31685 Liters = 0.02831685 m^3

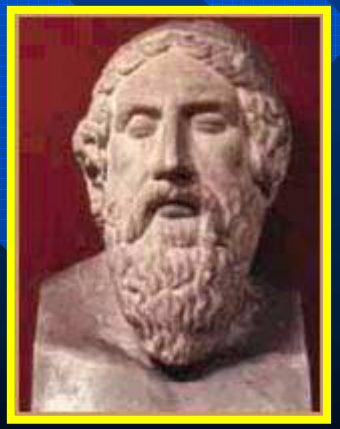
Microbiological Study of Micro-particulates

Fifth Plague of Egypt – ***"Murrain of beasts" Exodus 9:6***

Sixth Plague of Egypt – **"And it shall become small dust in all the land of Egypt, and shall be a boil breaking forth [with] blains upon man, and upon beast, throughout all the land of Egypt". Exodus 9:10**

25 BC: Poet Virgil

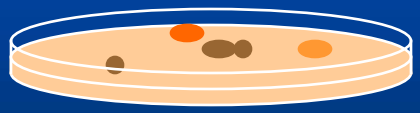
- ***The Iliad (Homer) "the burning wing of plague…"***
- ***Middle Ages: European pandemic "Black Bane" killed 60,000 cattle.***

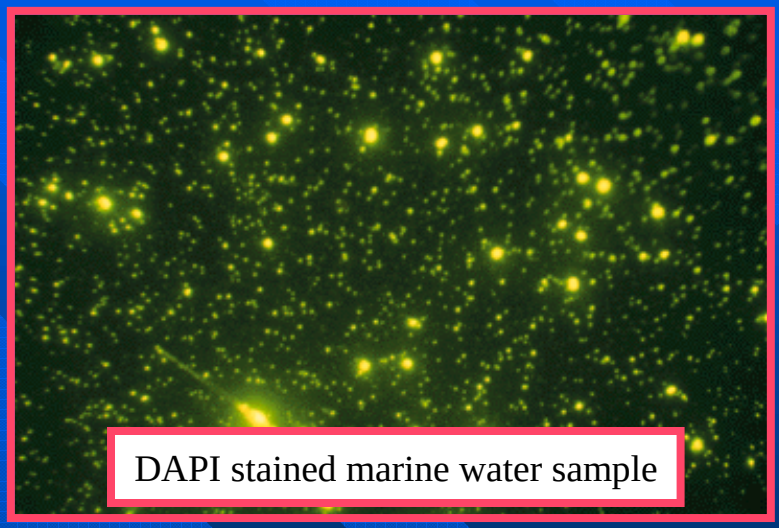


The great plate count anomaly

microbial community

plating

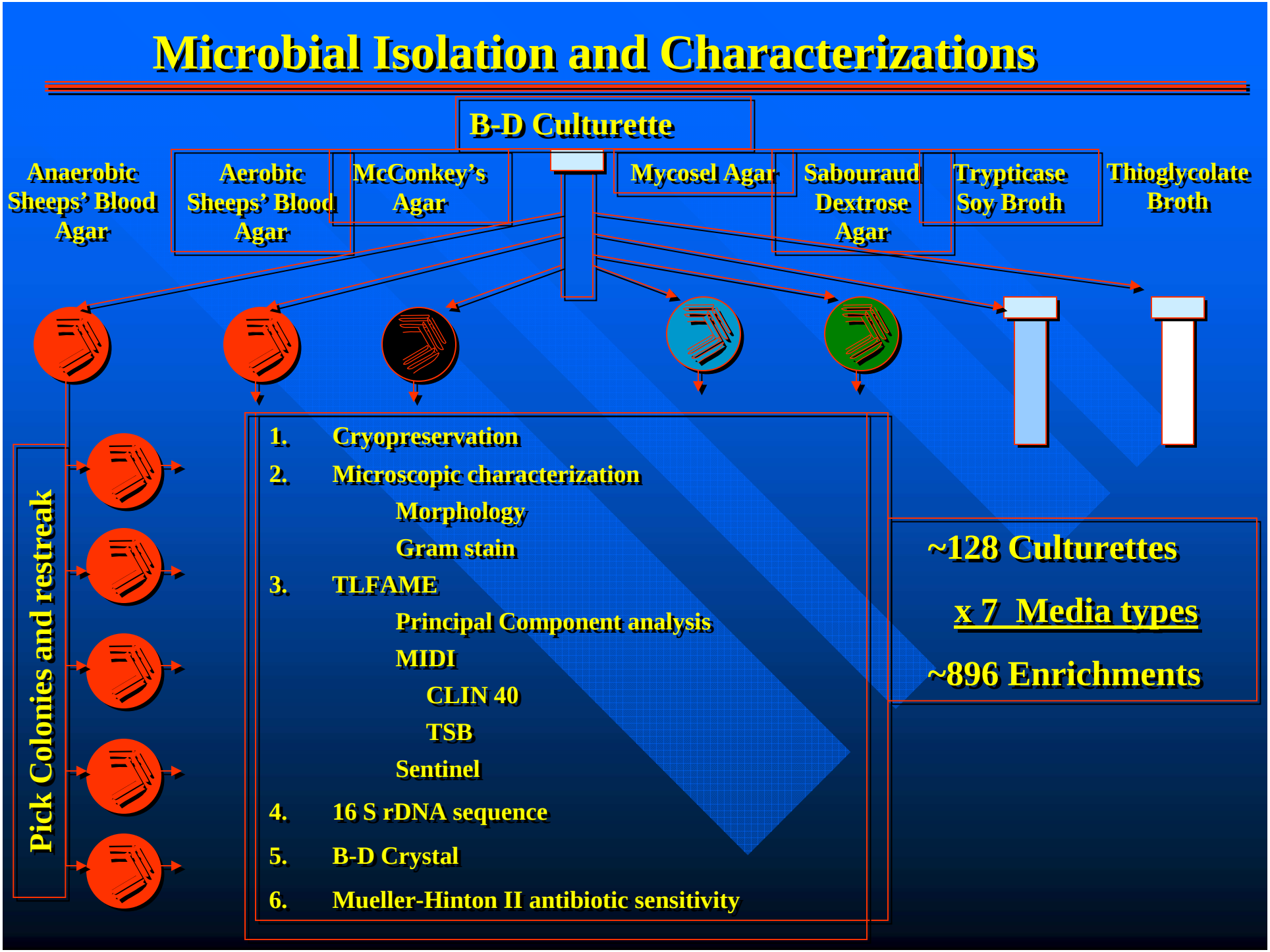




DAPI stained marine water sample

< 1% of observable bacteria grow on standard culture media

Microbial Isolation and Characterizations



Microbiology Summary

N0.	Site	Hemolysis on Blood aga	MIDI @ DE Environmental Database ID	imilarit Index	MIDI @ MS CLIN 40 Database ID	imilarit Index	MIDI @ MS Sentenial Database ID	imilarit Index	MIDI 500 bp rDNA sequnce analysis	% Difference		Best ID thus Far
2	Babylon	No	Pseudomonas stutzeri	0.597	Pseudomonas stutzeri	0.503	**Neisseria meningitis**	0.357				***Neisseria meningitis***
							Neisseria meningitis	0.29				
							Neisseria cinerea	0.29				
5	Babylon	No	Staphylococcus epidermidis	0.827	Staphylococcus aureus	0.676	**Staphylococcus aureus**	0.609				***Staphylococcus aureus***
			Staphylococcus epidermidis	0.78	Staphylococcus warneri	0.596	Staphyloccoccus epiderimidis	0.576				
			Staphylococcus capitis	0.753	Staphylococcus aureus	0.569	Staphyloccoccus hominis	0.497				
8	Babylon	No	**Bacillus circulans**	0.61	No match/Too dilute	N/A	No match					***Bacillus circulans***
9	Udairi	Alpha hemoly	Not sent to MIDI	N/A	Not Extracted	N/A						*None*
10	Udairi	Alpha hemoly	Ewingella americana	0.778	Neisseria cinera	0.204	Providencia rettgeri	0.023	**Pantoea agglomerans**	0.95%	Species	***Pantoea agglomerans***
			Salmonella typhimurium	0.592	Aeromonas veronii	0.175	Arcobacter skirrowii	0.018				
			Pantoea agglomerans	0.568	Neisseria cinera	0.169	Erwinia amylovora	0.017				
11	Udairi	Alpha hemoly	Pseudomonas stutzeri	0.896	Not Extracted	N/A			**Pseudomonas agrici**	1.34%	Genus	***Pseudomonas agrici***
			Pseudomonas balearica	0.659								
			Pseudomonas resinovorans	0.584								
12	Udairi	No	**Not growing when others s**	N/A	Vibrio alginolyticus	0.366	**Ralstonia paucula**	0.127				***Ralstonia paucula***
					Aeromonas hydrophilia	0.366	Erwinia mallotivora	0.103				
					Neisseria mucosa	0.335	Ralstonia basilensis	0.1				
14	Udairi	No	**Not growing when others s**	N/A	Staphyloccoccus epiderimidis	0.419	**Staphylococcus pasteuri**	0.207				***Staphylococcus pasteuri***
							Staphylococcus caprae	0.185				
							Staphylococcus warneri	0.135				
15	Udairi	No	Virgibacillus pantothenticus	0.677	Bacillus coagulans	0.432	Arthrobacter atrocyaneus	0.414	**Arthrobacter crystallopoietes**	0.00%	Species	***Arthrobacter crystallopoietes***
			Micrococcus luteus	0.499	Dermobacter hominis	0.327	Agromyces ramosus	0.283				
			Bacillus atropheus	0.477	Kocuria-varians(Micrococcus)	0.316						
16	Udairi	No	Staphylococcus warneri	0.881	Pseudomonas stutzeri	0.44	**Pseudomonas balearica**	0.097				***Pseudomonas balearica***
			Staphylococcus epidermidis	0.754	N/A	N/A						
			Staphylococcus epidermidis	0.61	N/A	N/A						
17	Udairi	Beta/Alpha	Paenibacillus thiaminolyticus	0.534	Not Extracted	N/A			**Paenibacillus** thiaminolyticus	2.97%	Genus	***Paenibacillus*** *thiaminolyticus*
			Bacillus atrophaeus	0.464								
18	Udairi	Beta hemolyti	Bacillus subtilis	0.901	Bacillus subtilis	0.52	**Bacillus vedderi**	0.656				***Bacillus vedderi***
			Bacillus atrophaeus	0.697	N/A	N/A	Bacillus mojavensis	0.642				

Summary of Soil Isolates

Best ID thus Far	Comment
Neisseria meningitidis	meningitis
Staphylococcus aureus	cystic fibrosis
Bacillus circulans	gastro-enteritis
NONE	Unidentified
Pantoea agglomerans	septic arthritis
Pseudomonas agrici	
Ralstonia paucula	opportunist-septicemia, peritonitis, abscesses
Staphylococcus pasteuri	various infections
Arthrobacter crystallopoietes	
Pseudomonas balearica	cystic fibrosis
Paenibacillus thiaminolyticus	bacteremia
Bacillus vedderi	obligate alkaliphile
Bacillus subtilis	
Pantoea agglomerans	epiphyte
Pseudomonas pseudoalcaligenes	
Cryptococcus albidus	septicemia and meningitis
Bacillus clausii	Oral bacteriotherapy
Kurthia gibsonii	Diarrhorea
Bacillus firmus	alkaliphile; bread spoilage
Staphylococcus kloosii	various infections
Bacillus mojavensis	biosurfactant
Bacillus licheniformis	food poisoning
Pseudomonas oryzihabitans	Hickman catherter biofilm

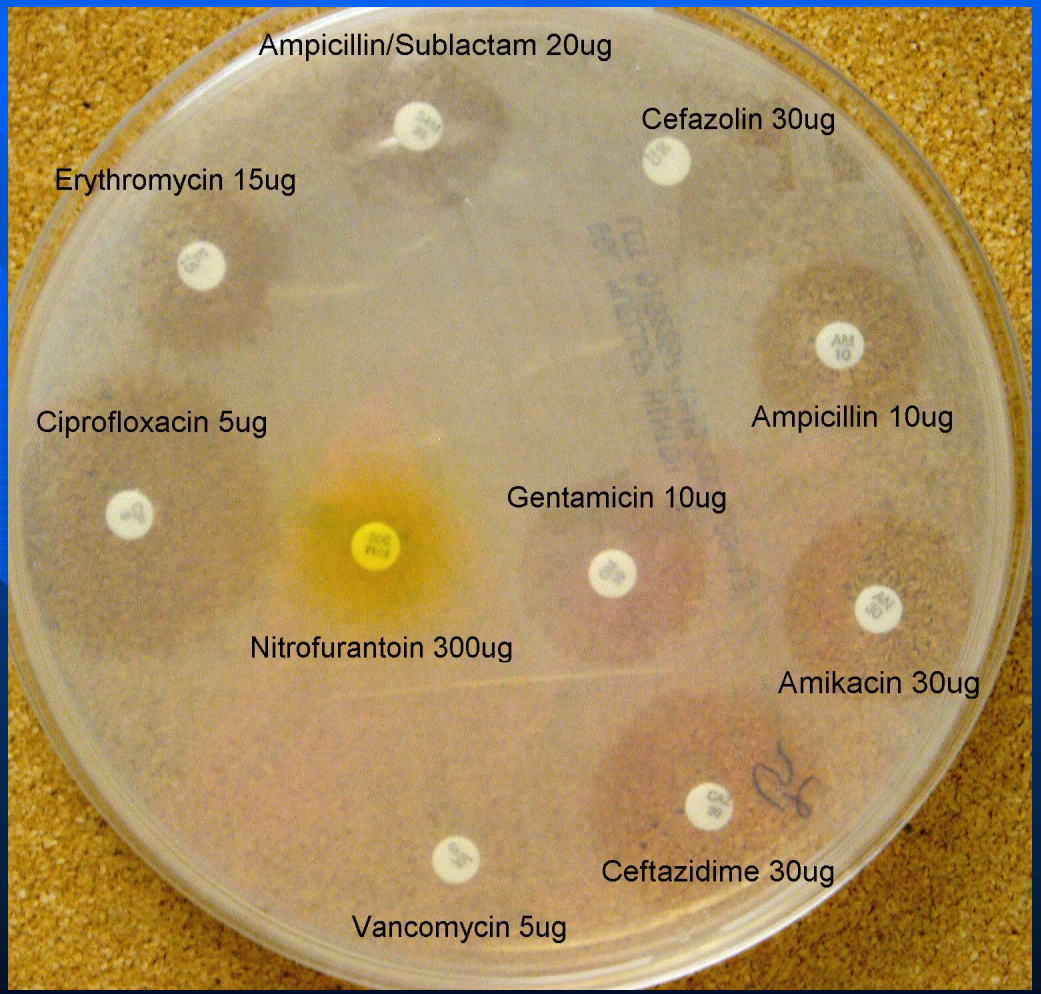
B-D Crystal Biochemical Characterization of Hemolytic Isolates

Sample	Gram + Organism	Confidence	Associated Disease
#25	Kytococcus sedentarius	0.467	Pitting of human epidermis in keratolysis
#36	Kocuria rosea	0.9927	Catheter related bacteremia
#72	No Match	*	
#56	Gardnerella vaginalis	0.9982	Human bacterial vaginosis
#69	Leifsonia aquaticum	0.9999	Rare bacteremia
#22	Leifsonia aquaticum	0.9941	Rare bacteremia
#16	Corynebacterium pseudotuberculosis	0.6877	Ventral lymphadenitis, abscesses, and ulcerative dermatitis in cattle
#70	Leifsonia aquaticum	0.9951	Rare bacteremia

Sample	Gram neg. Organism	Confidence	Comments
#22	No Match	*	Additional testing required to establish ID
#16	No Match	*	Additional testing required to establish ID
#36	Yersinia pestis	0.9384	*
#25	Pseudomonas stutzeri	0.8598	Supplemental testing recommended
#69	No Match	*	Additional testing required to establish ID
#72	No Match	*	Additional testing required to establish ID
#56	No Match	*	Additional testing required to establish ID
#70	No Match	*	Additional testing required to establish ID

Antibiotic Sensitivity

Beta Hemolytic Isolate Number 69



Bacteria Isolated from Kuwait and Iraq that have Shown Antibiotic Resistance.

Culture#	Description	Location	Culturette	Hemolysis	Colony Morphology	MIDI @ DE Environmental	Similarity Index	Comment
8	BSSI	Babylon	Green	No	Dry Fungal type colonies; White spreader on TSA & Blood	Bacillus circulans	0.61	N/A
12	>20<44 *u*m	Udairi	Green	No	Cream colored mucoid colonies on Blood and TSA;	**Not growing when others sent off**	N/A	N/A
16	>44<63 um	Udairi	Green	No	Small mucoid colonies on Blood; Spreading mucoid on TSA;	Staphylococcus warneri	0.881	N/A
20	>44<90 um	Udairi	Green	alpha	Small dry cream colored colonies	Pantoea agglomerans	0.82	GC subgroupB
						Pantoea agglomerans	0.711	GC subgroupC
24	<20 um	Udairi	Green	No	Shiny yellowish/cream spreading colonies on TSA; Purple spreader on Blood;			
28	TAB II Sand A	Tallil	Green	No	Large shiny mucoid colonies	Not sent to MIDI	N/A	N/A
32	TAB II Sand B	Tallil	Green	beta	Clear white cauliflower colony on TSA; Shiny clear runny colony on Blood;	Not sent to MIDI	N/A	N/A

Culture#	MIDI @ MS CLIN 40	Similarity Index	Comment	MIDI @ MS Sentenial	Similarity Index	MIDI 500 bp rDNA sequnce analysis	% Diff	Comments
8	No match/Too dilute	N/A	N/A	No match				
12	Vibrio alginolyticus	0.366	N/A	Ralstonia paucula	0.127			
	Aeromonas hydrophilia	0.366	N/A	Erwinia mallotivora	0.103			
16	Pseudomonas stutzeri	0.44	N/A	Pseudomonas balearica	0.097			
20	Pantoe aggomerans	0.623	GCsubgroup	Ralstonia paucula	0.274	Pantoea agglomerans	0.85%	Plant/Human
	Aeromonas hydrophilia	0.386	N/A	Buttiauxella gaviniae	0.175			Pathogen
24								
28	Not Extracted	N/A	N/A					
32	Not Extracted	N/A	N/A			Flavimonas oryzihabitans	0.10%	Hickman Cath. Pathogen

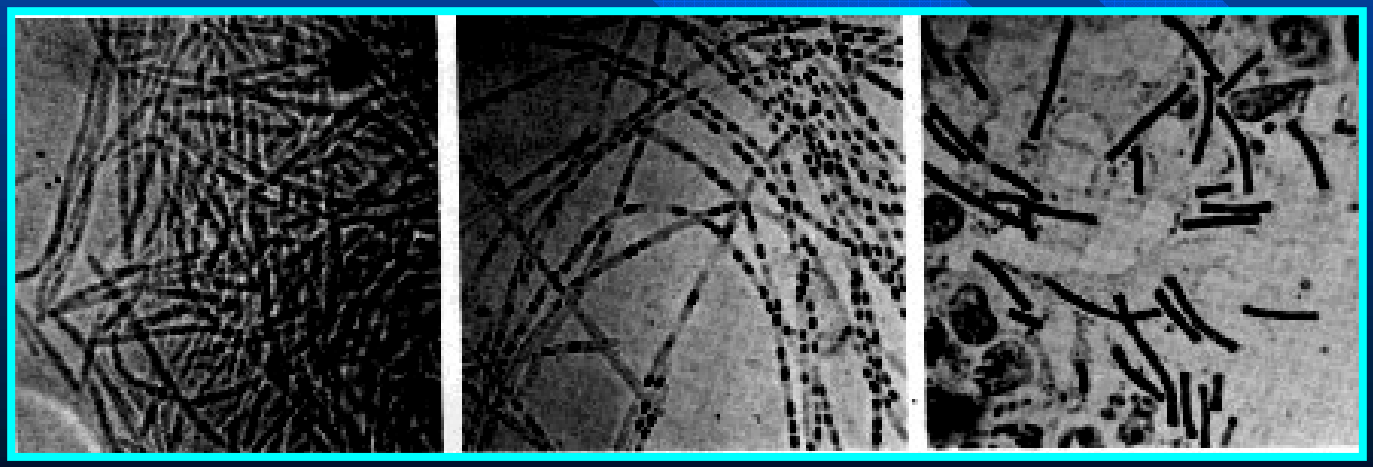
Fungal Isolates

~300 bp of D2 region of LSU rDNA

Microseq Library database								
Midi D2(300 bp)LSU rRNA	**% diff**	**LSU D2 Genbank Database**	**% ID**	**Associated Disease**				
Allewia eureka	0.31	Ulocladium sp.	99					
Allewia eureka	4.64	Cryptococcus uzbekistanensis	100					
Allewia eureka	0.31	Ulocladium sp.	99	None				
Altemaria altemata	0	Alternaria sp	100	Plant pathogen				
Rhodotorula minuata	5.73	Rhodotorula minuata	99	Eye infections				
Cryptococcus albidus	2.44	Cryptococcus sp.	100	Cryptococcus neoformans - meningoencephalitis				
Ulocladium chartarum	0.31	Stemphylium sp	99	Fungal biocontrol agent				
Filobasidium uniguttulatum	4.64	Cryptococcus uzbekistanensis	100	Teleomorph of Crytococcus, non pathogenic yeast				
Ulocladium consortiale	0	Stemphylium sp	100	cutaneous mycoses				
Ulocladium chartarum	0.31	Stemphylium sp	99					
Mortierella polycephala	7.1	Mortierella polycephala	92	Pulmonary mycosis in cattle				
Embellisia chlamydospora	0	Ulocladium sp.	99	none				
Filobasidium uniguttulatum	4.64	Cryptococcus uzbekistanensis	100					
Penicillium camembertii	0	Penicillium sp.	100					
Cryptococcus albidus	0	Cryptococcus albidus	100					
Allewia eureka	0.31	Ulocladium sp.	99					
Embellisia chlamydospora	0	Ulocladium sp.	99					
Filobasidium uniguttulatum	4.64	Cryptococcus uzbekistanensis	100					
Embellisia chlamydospora	0	Ulocladium sp.	99					
Filobasidium uniguttulatum	4.64	Cryptococcus uzbekistanensis	100					
Penicillium camembertii	0	Penicillium sp.	100					
Allewia eureka	0.31	Ulocladium sp.	99	Plant pathogen				
Phoma glomerata	0	Phoma herbarum	99					

Summary of the Biological Analysis

- *Hemolytic microorganisms*
- *Gram positive spore-formers*
- *Gram negative opportunists*
- *Fungi*



Conclusions Thus Far

- Relatively abundant microbial community in dust
  - Including $PM_{2.5}$
- Hemolytic strains isolated/characterized
- Microbial identification systems cause uncertainty
  - B-D Crystal biochemical characterization
  - TLFAME – MIDI CLIN 40, Sentinel TS, MIDI Env
- Methods bias perspective of microbial community
- Need a defensible trigger for concern