Airborne Mineral Dust Aerobiology and Health in North Africa

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Presentation Goals

- Highlight
  - Studies of the atmospheric microbiome in African air masses
  - The role of aerobiology in health and food security
  - Relevance of aerobiology as an Air Quality component

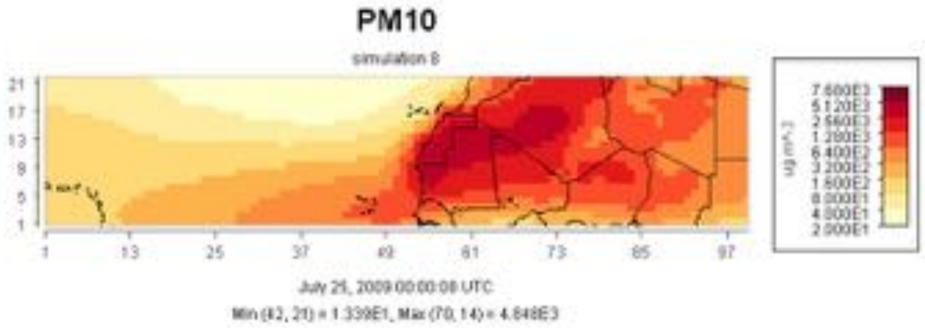
Outline

- Introduction
- Background Work
- Inspirations for Study, Study aims and Hypotheses
- Methodologies
- Summary of Results, Key Events, and Challenges
- Future Directions and Relevance to Air Quality
- Acknowledgements

Broad Science Questions

- What type of bacteria are generally present in the air and what are their associations with aerosol distributions in each site of interest?
- What are the meteorological or climate controls on airborne microbes (viability and activity)? Are there feedback effects?
- How does air mass origin and aging (aerosol life cycle) affect endpoint microbial distributions?
- What are the observed transport and deposition patterns of the various microbial species?
- How do specific airborne microbial communities contribute to public and environmental health?
- What is the diurnal and seasonal variability inherent in the types of bacteria during the observation periods?

A Variety of Infectious Bacteria Easily Spread Through the Air.



- *Mycetoma*
- *Bordetella pertussis*
- *Clostridium diphtheriae*
- *Mycobacterium tuberculosis*
- *Neisseria meningitis*
- *Staphylococcus aureus*
- *Yersinia pestis*
- *Haemophilus infuenzae*
- Skin pathogens from the *Staphylococcus* and *Streptococcus* species.

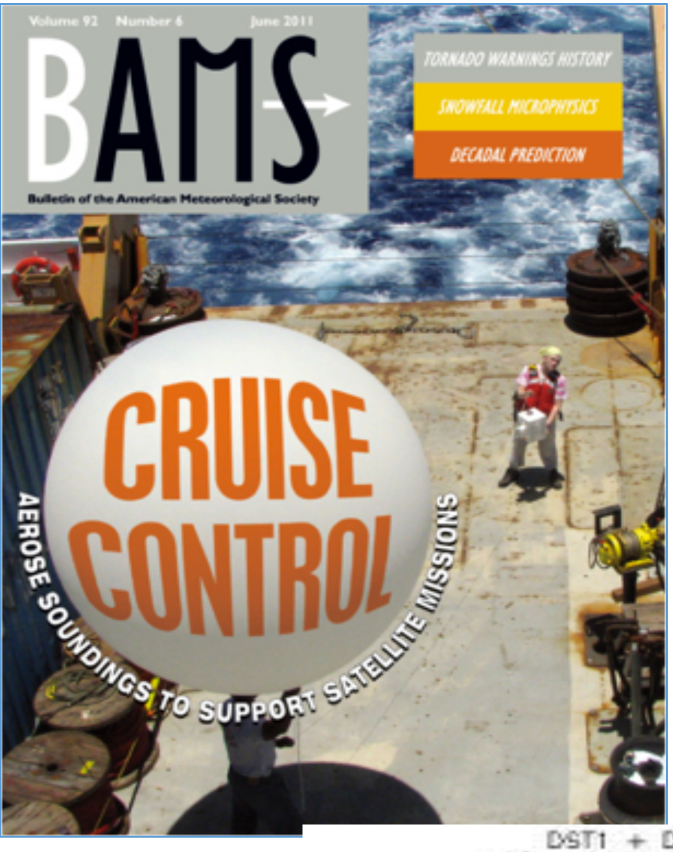
Key Questions and Testable Hypotheses

- Is there significant microbiology on airborne particulate?
- Are there significant changes in the microbiological nature of airborne particulate across the wet season-dry season transition?
- Are there changes related to the chemistry of the particulate
- Do the chemical or biological properties of the particulate influence health in the open air hospital environment?

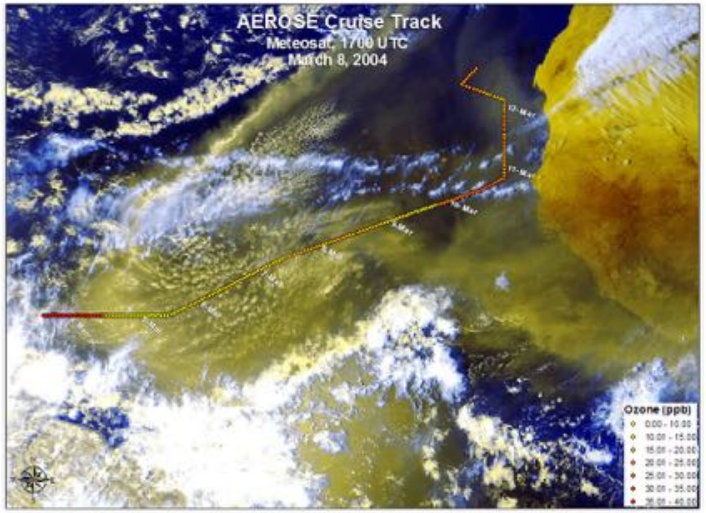
- Hypothesis: The microbiology will tend towards having a signature of air mass origin (source-specific)
- Hypothesis: The microbiology should reflect seasonal changes in both the source regions and receptor regions.
- Hypothesis. The chemistry of the atmospheric particulate (in particular geogenic materials) will evolve during transport
- Hypothesis. The chemical environment provided by the particulate may influence microbial distributions.

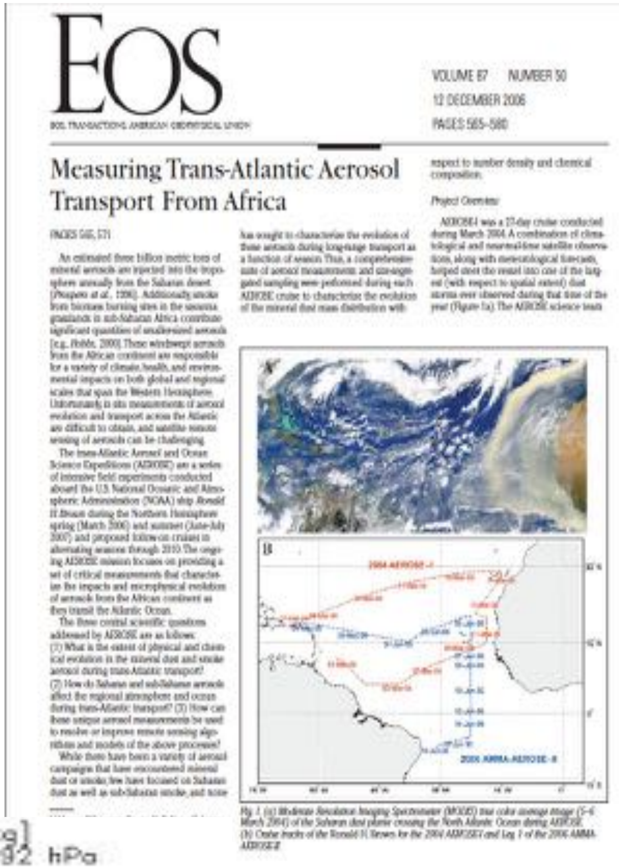
The current project builds upon work performed over the past several years

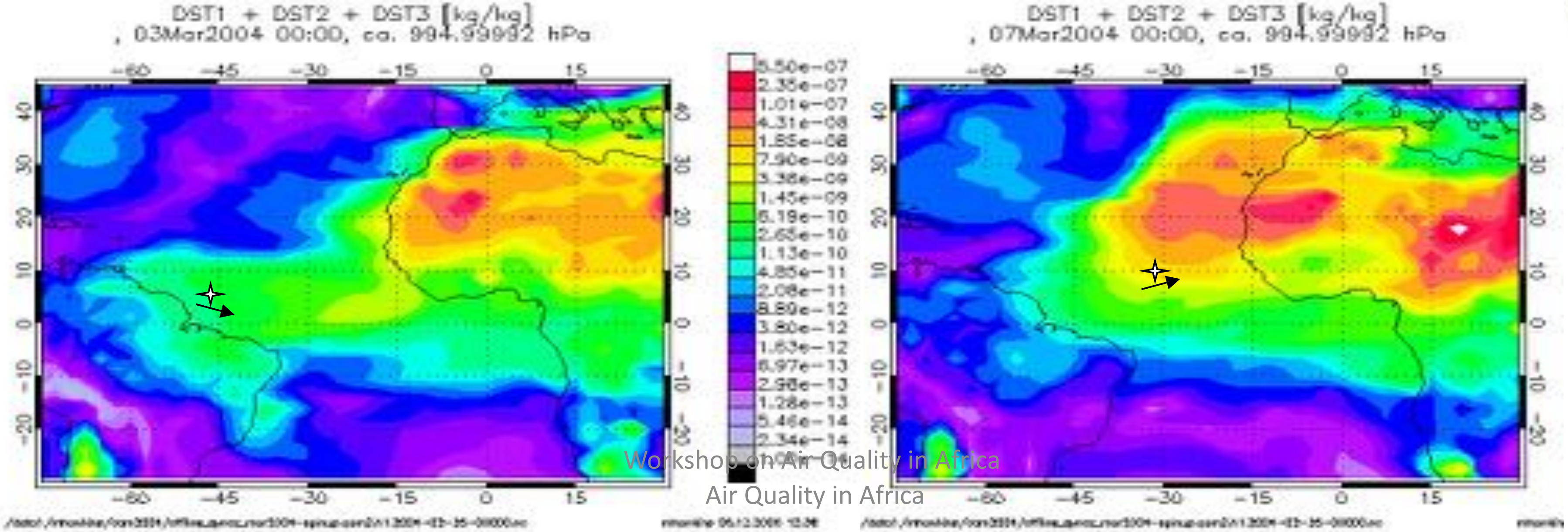
- Fungal diversity associated with trans-Atlantic Saharan dust storms (2004 – present)
- Evaluation of culture-based methods to investigate microbial diversity in urban aerosols in Washington, DC (2006 – present)
- Comparison of culture-based and genomic methods for analysis of microbial communities in Bamako, Mali (2006 – 2007)
- Evaluation of microbial communities in trans-Atlantic Dust storms (2009 – present)
- Fungal diversity and food security implications of trans-Atlantic Saharan dust storms (2011 – present)
- Bio-aerosol characterization in SE Asian megacities (2010 – present)
- Microbial diversity in global soils relevant to fugitive dust transport (2013 – present)



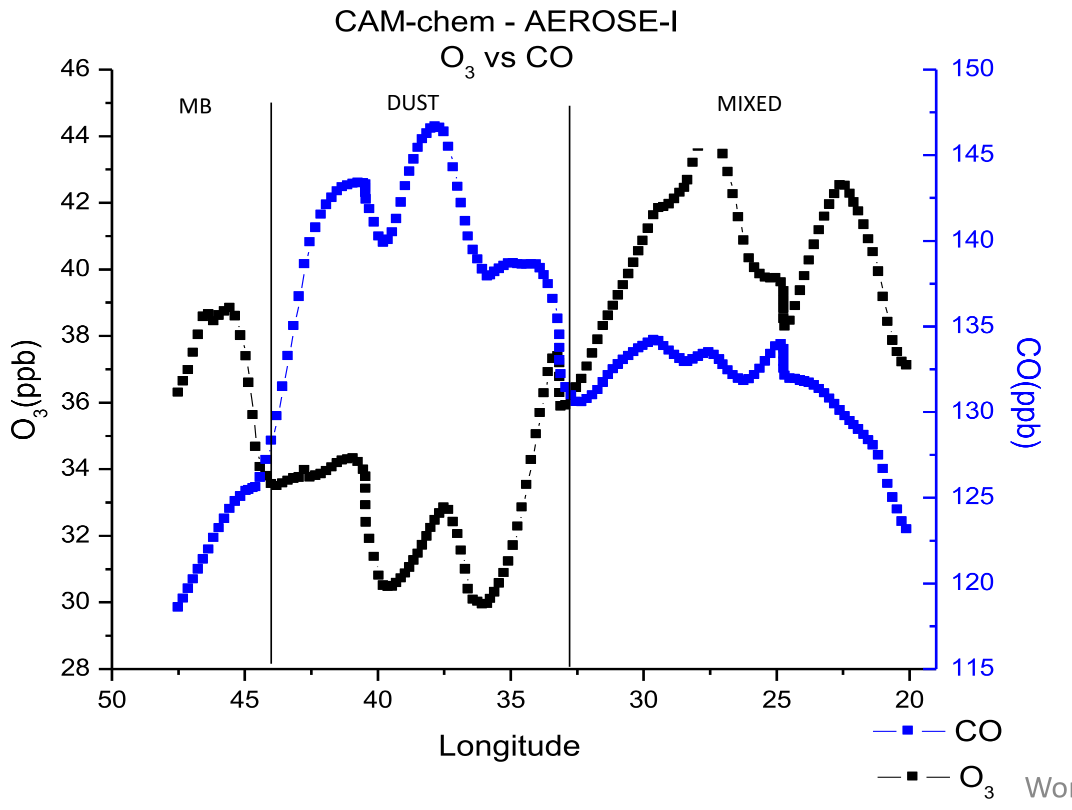
Over a decade of in situ observations of Saharan Aerosol Transport and Evolution in the Tropical Atlantic

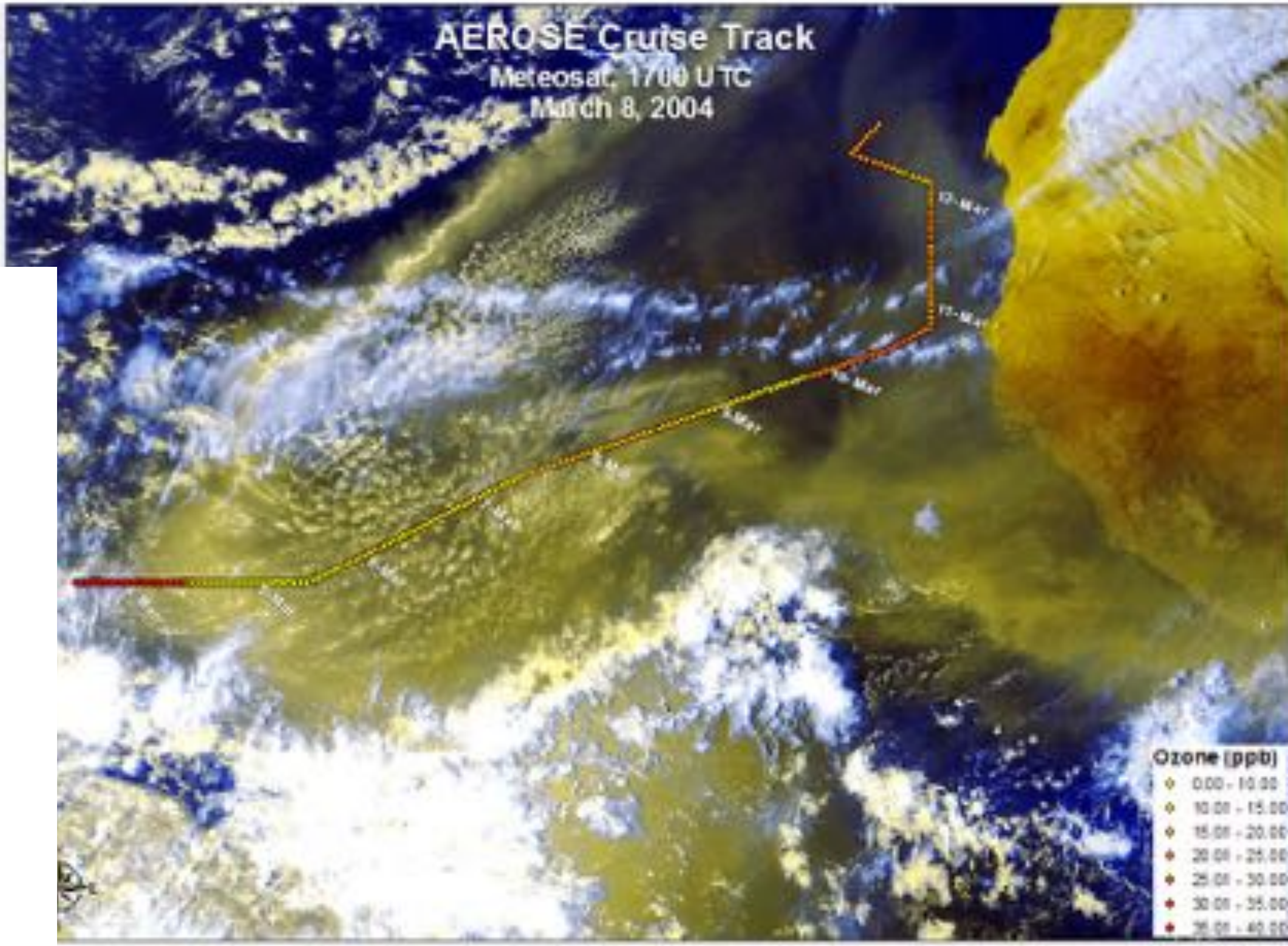




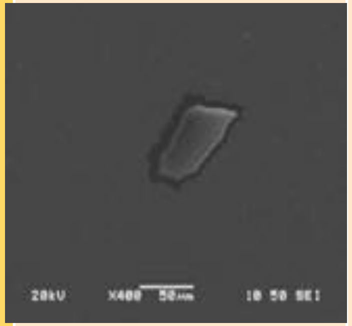
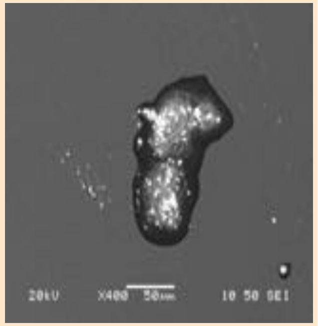
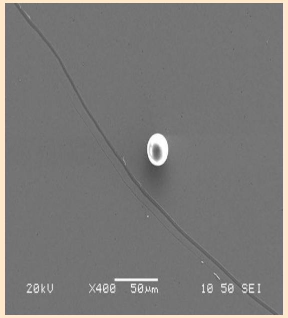
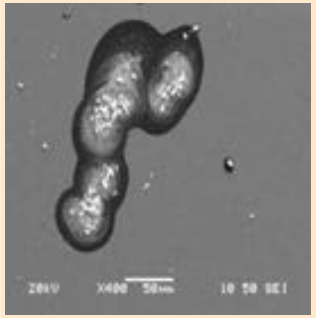
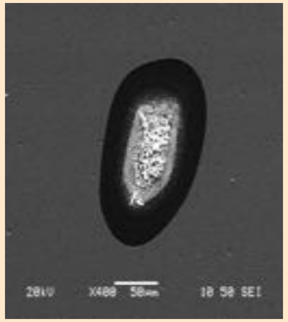
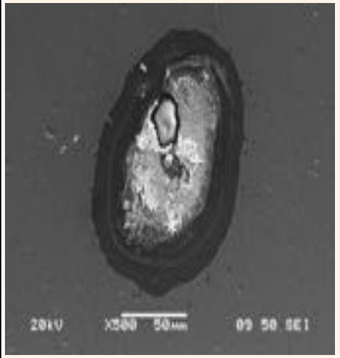
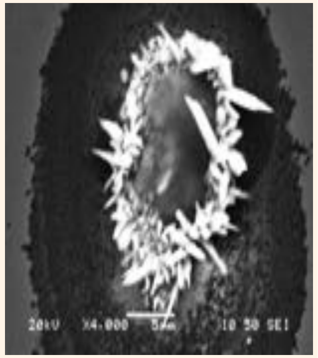
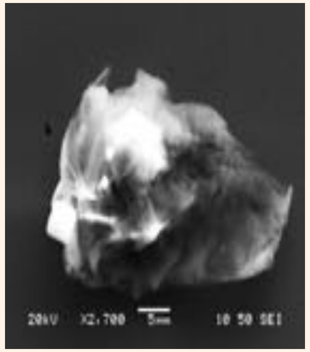
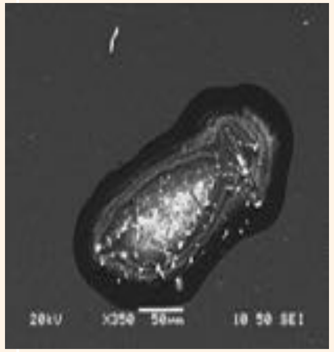
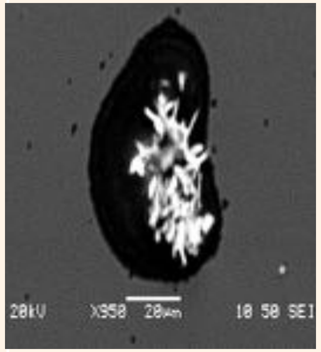


In situ observations for verification of Chemistry and Transport Models and Chemical Mechanisms for Global Models

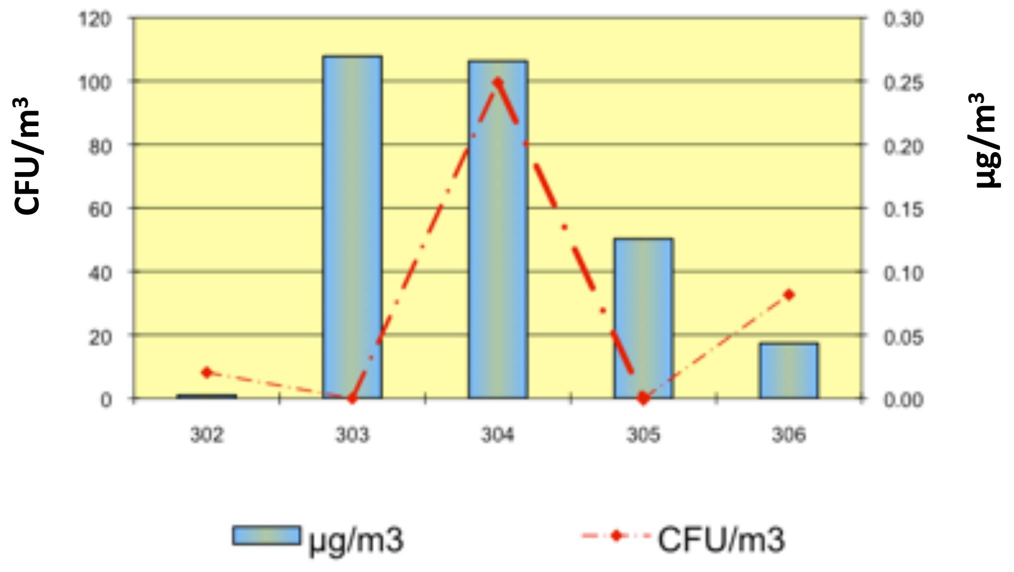


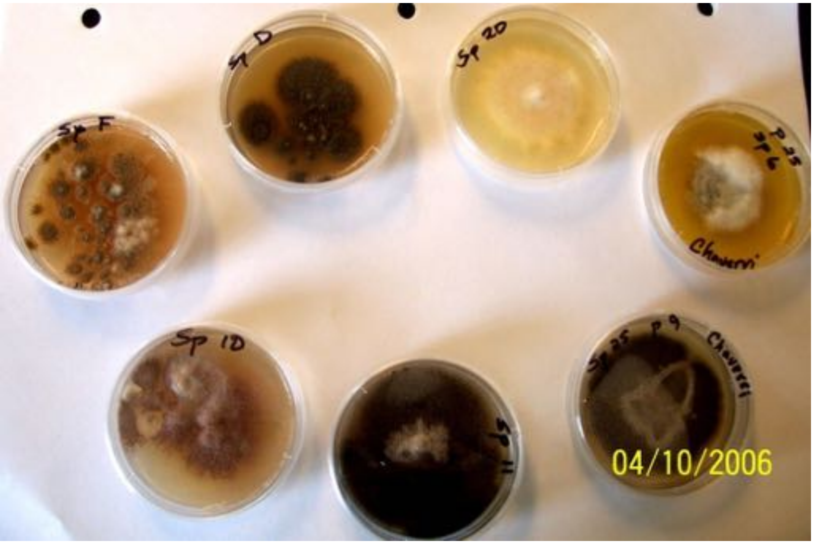


Atmospheric Particulate Serve as Efficient Vehicles for Pathogen Transport

Size, µm / Date	5.0	2.5	1.2	0.6	0.3	0.15
07/04/06						
07/10/06						

Identification of Fungi in Saharan Dust





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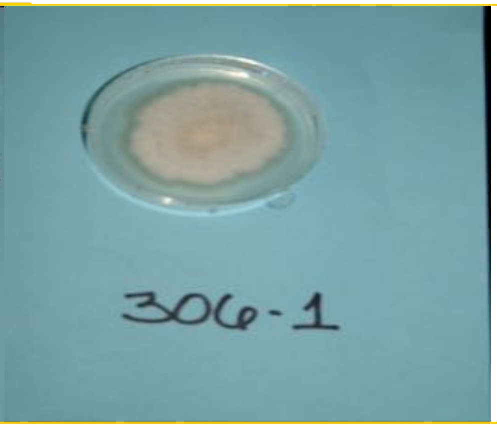


Salal Root
Associated Fungi

Aspergillus fumigatus
Penicillium chrysogenum
Thielavia fragilis

Thielavia subthermophila

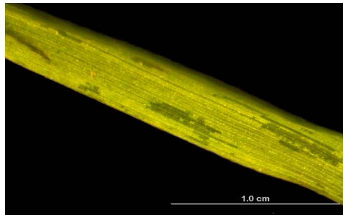
Results From Intercontinental Transport of Fungi – AEROSE Environmental Samples (454 Pyrosequencing)

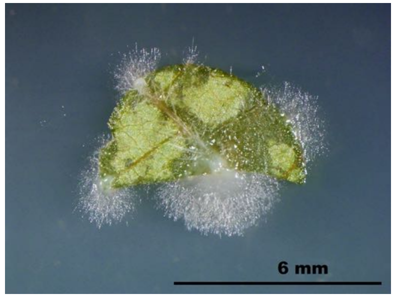
Most abundant species, 2009

Trichoderma cf. harzianum	**19751**	**Mycoparasite**	
Trichoderma reesei	**11807**	**Saprophyte / rarely human pathogen**	**Wide range**
Humicola fuscoatra var. fuscoatra	**2836**	**Pathogen - Roots**	**Tomato plants**
Bionectria ochroleuca	**1612**	**Mycoparasite**	**fungi/insects**
Botryosphaeria viticola	**1236**	**Pathogen**	**Vitis vinifera**
Monochaetia sp. 162	**444**	**Pathogen/canker**	**Apples (if *M. mali*)**
Madurella mycetomatis	**410**	**Pathogen/mycetoma**	**Humans**
Endothia sp. IFB-E023	**348**	**Pathogen/dieback**	**wood**
Fusarium sp. BI	**179**	**Pathogen**	**Wide range**
Discostroma botan	**140**	**Pathogen/stem lessions**	**Paeonia suffruticosa**
Botryosphaeria ribis	**102**	**Pathogen/canker, dieback**	

Food Security Implications

- ~80% percent of the sample is dominated by pathogens or mycoparasites (species with biocontrol potential
- 5-6 fold increase in species diversity with the dominant species represent about 10% of the total species diversity)
- Observed an as yet undiscovered inter-annual dynamic diversity of species.
- Wheat and Soy are the most susceptible cash crops
- Bacterial pathogens species: gram positive and gram negative, *Xanthomonas*
- Fungal pathogens identified:*Sclerotinia* sp., *Fusarium* sp.

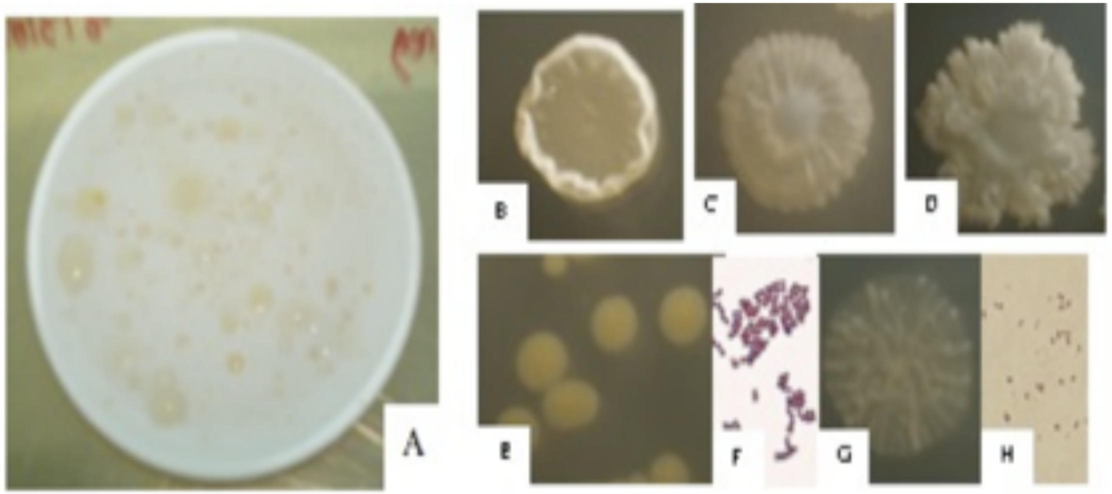




Aerobiology in Mali

Phenotypic Characterization

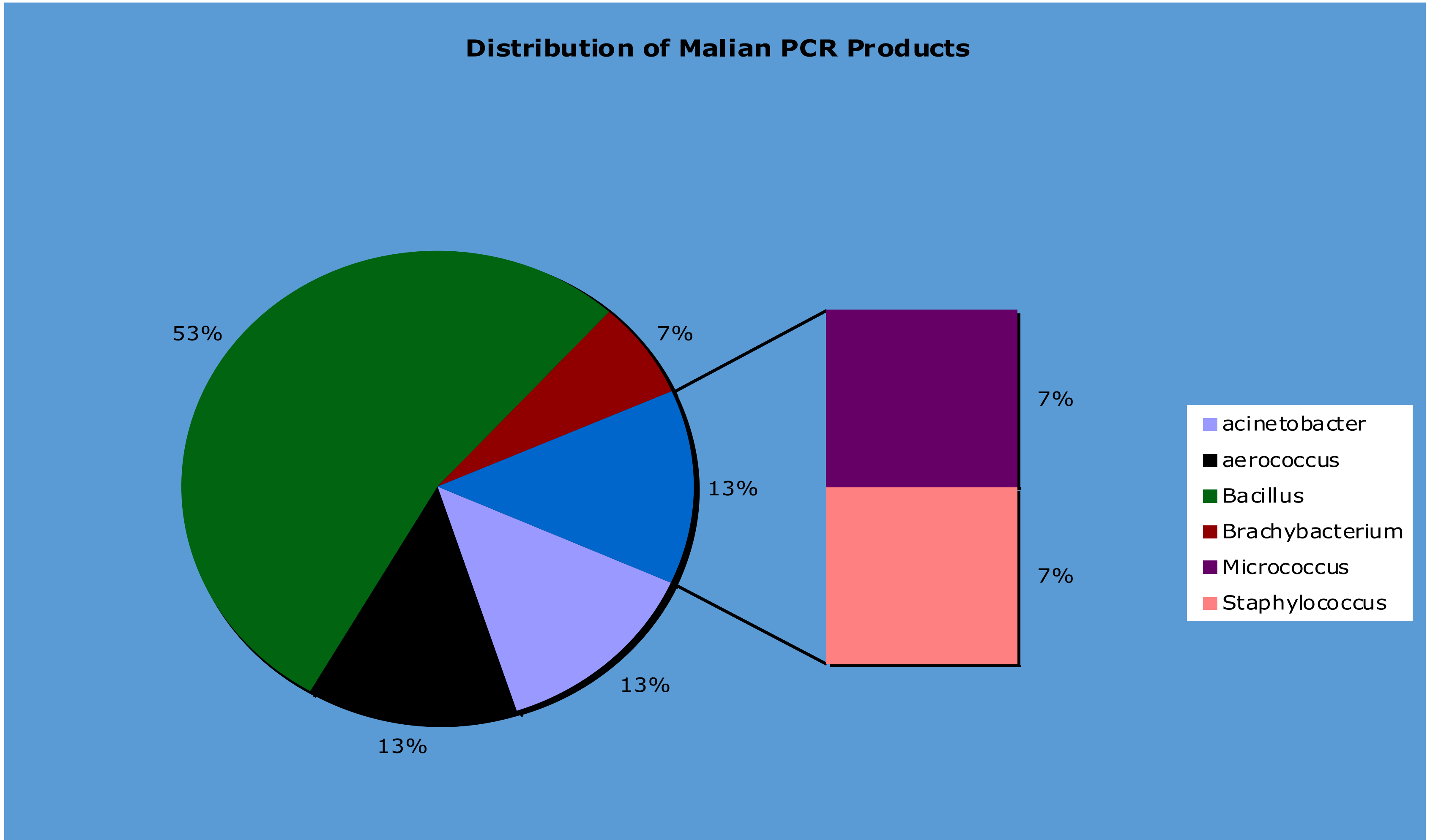
- Mali (July-Aug '07)
  - 46-195 CFUs
  - Total of 115 bacterial isolates
    - 83% Gram positive, 70% spore-formers
- Mali (Oct '07)
  - 71-185 CFUs
  - Total of 107
    - 82% Gram positive



Cultured filter sample, isolated colonies and microscopic characteristics

(A): Microbial growth after 24hr incubation in BHI agar. (B, C, D, E and G): Microbial colonies visualized by stereometer. Microscopic photographs of gram stained (F) cocci tetrads and (H) Rods

Less than 40% of airborne species are readily identifiable by molecular techniques



So… what’s happening in Ethiopia?

Original Inspirations for the Study

Climate and Aerosol Dynamics

- How do the current monsoonal transitions (2014 -2016) compare to the climatological record?
- How are variations in the seasonal transition connected to air mass trajectories?
- How does air mass origin and aging (aerosol life cycle) affect endpoint microbial distributions?
- What is the diurnal and seasonal variability inherent in the types of bacteria during the observation periods?

Environmental Microbiology

- What is the seasonality of the atmospheric microbiome in the Ethiopian Highlands Region?
- What are the associations between population dynamics of the atmospheric microbiome and precipitation?
- What type of bacteria are generally present in the air and what are their associations with aerosol distributions in each site of interest?
- What are the associations between population dynamics of the atmospheric microbiome and air mass origin?

AACAM Goals for Understanding at Gondar Medical Campus

- To help in the identification of bacterial sources bearing in mind that air masses can move viable pathogens both regionally and globally.
- To identify bacterial species able to infect health and health-impaired individuals
- To determine variations in the type of airborne pathogens with the changes in the environmental conditions.

Specific Activities

Ambient Aerosol Chemistry and the Atmospheric Microbiome

- Primary Project Elements
  - Limited diurnal profiling with inline filter - microbiology only
  - Outdoor PM sampling - Staplex 3-7 filters
    MCE and Polycarbonate filters for microbiology
    MCE, PTFE, and Quartz filters for chemistry
    Atmospheric Modeling and Meteorological Analysis
  - Culturing and DNA Isolation
  - Phenotypic analysis
  - Statistical data analysis

Indoor Air Quality of selected wards on the Gondar Medical Campus

- Primary Project Elements
  - Designation of Leishmaniasis, Tuberculosis, and ICU Wards for indoor AQ
  - Staplex™ sampling periods - 3 filters comprising fine mode aerosols
  - Culturing and Phenotypic analysis of the microbial communities on the size-segregated samples
  - Isolation of the DNA obtained from the pure cultures
  - 16S rDNA Sequencing of the isolates
  - Comparison to patient data collected in clinical trials

Sampling Approach

Instrumentation

- ✓ Sampling Methods (Quartz fiber filters or Quartz crystals)
- ✓ In-situ aerosol number densities were measured using laser particle counters (Climet, TSI Dust Track)
- ✓ Microbial Samples were collected using a Staplex microbial sampler

Publications

- A. L. Northcross, S. Hsieh, S. Wilson, E. Roper, R. Dickerson, and **V. Morris** Monitoring Neighborhood Concentrations of PM2.5 and Black Carbon: When Citywide Averages Average Out Hotspots [Submitted to Environmental Monitoring and Assessment, 2016]
- M. A. Velez-Quinones, B. Eribo, K. E. Nelson, G. A. Nunez, and **V. R. Morris** Analysis of Viable Airborne Bacteria in Ambient Aerosols of Bamako, Mali: Potential Sources and Transport Patterns Under review Environmental Microbiology]
- A. D. Allen, B. Eribo, M. A. Velez-Quinones**, V. R. Morris** MALDI-TOF MA and 16SrRNA as Tools of the Evaluation of Bacterial Diversity in Soils from Sub-Saharan Africa and the Americas Aerobiologia 31:111-126, 2015
- S. Abegaz, N. Greene, **V. Morris** Spatio-Temporal Distributions of Particulate Matter Exposures in Washington, D.C. Journal of Natural and Environmental Sciences 2(1) 1 2011
- S. Abegaz, D. Raghavan, C. Hosten, **V. R. Morris** Evaluation of Heavy Metal Variability in Ambient Air in Washington, D.C. Environmental Pollution 155 (1) 88-98 2008.

Sampling Objectives

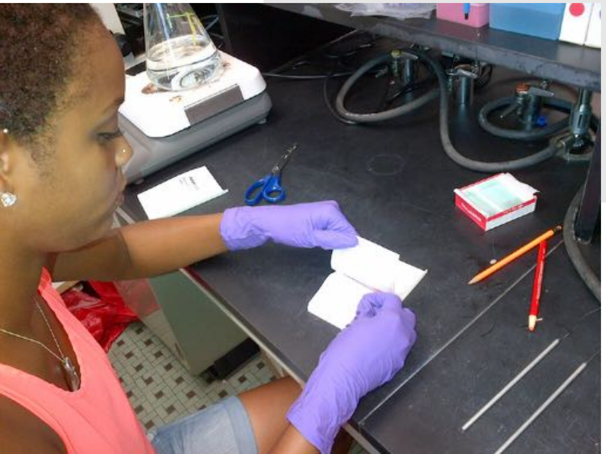
- Analyze the microbiology of ambient aerosols with respect to:
  - Aerosol Size Fraction
  - Culturing Nutrients
    - Reasoner’s 2A agar (R2A)
    - Trypticase soy agar (TSA)
  - Meteorological patterns
  - Chemical environment

- Evaluation of CFU dependence on TOD, air mass origin and type,

Microbiological Methods

Phenotypic Characterization

- Physiological and biochemical characterization of bacterial isolates
- Identifying nutritional requirements and optimal conditions required for growth. Primarily for viable culturable isolates



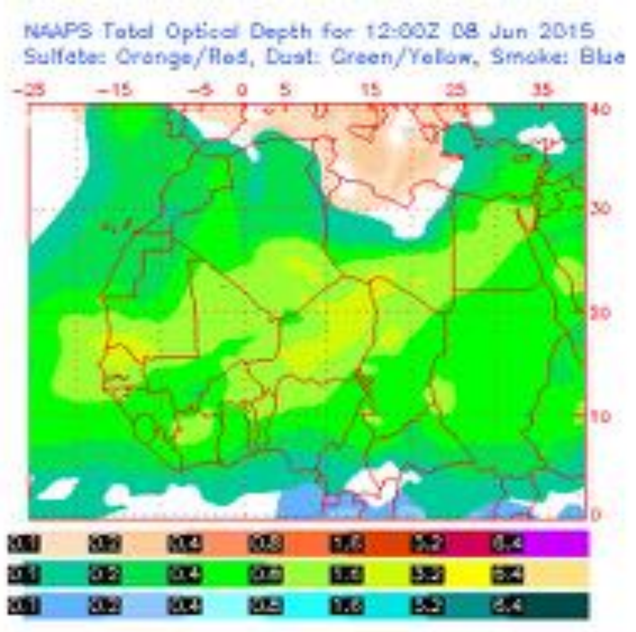
Molecular Characterization

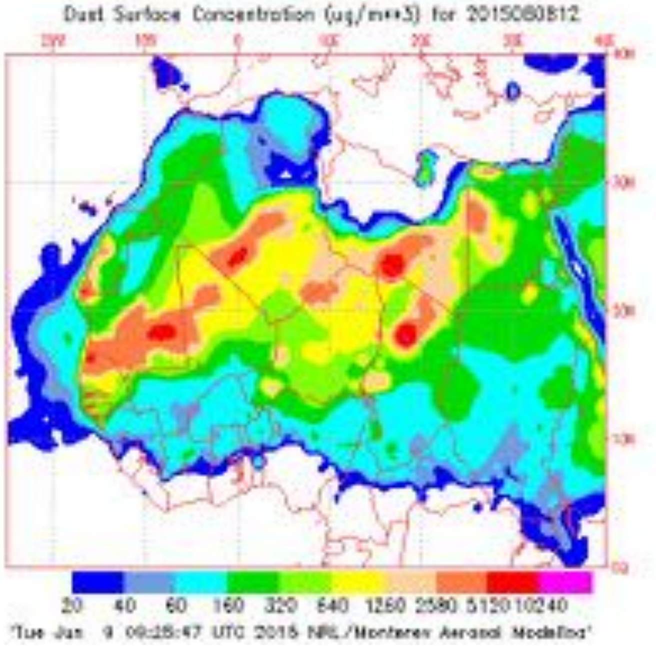
- 16S rRNA Sequencing; pyrosequencing etc.
- Complete bacterial diversity identification directly from the environment (no requirement for cultivation).
- Rapid and useful technique for detecting incidences of biological warfare.
- Does not differentiate viable from nonviable isolates

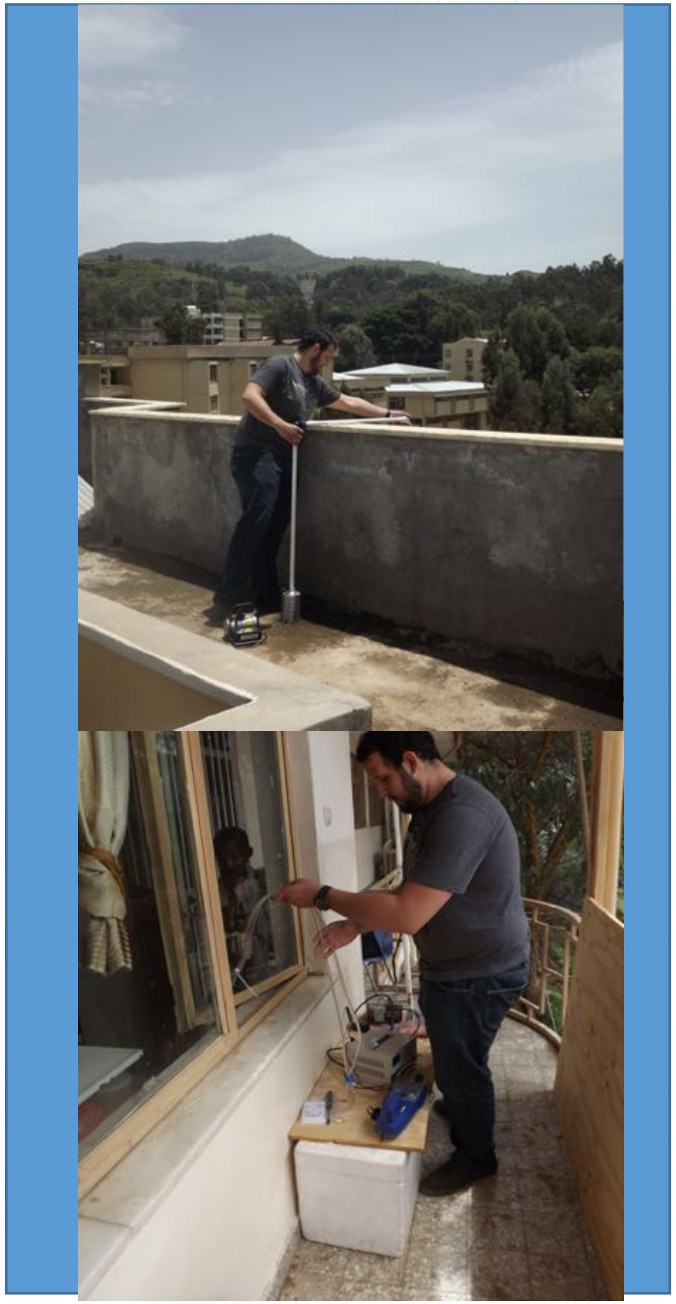
Evaluation of Virulence

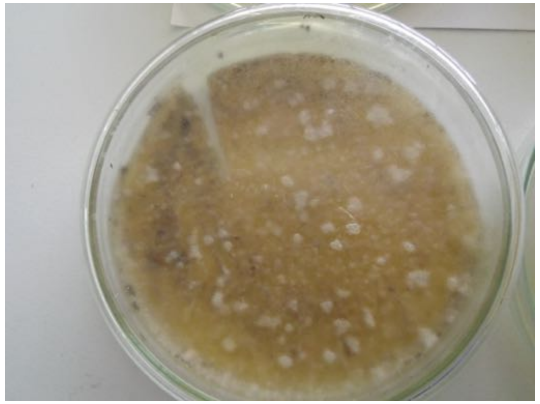
- All isolates
  - Peptide mass fingerprinting (PMF) + Phospholipid fatty acids (PLFA), Fatty acid methyl esters (FAME)
  - Specific virulence factors + PCR and sequencing
- Chromogenic isolates
  - the mechanism of action underlying various pigments/secondary metabolites in the survival/susceptibility of bacterivorous protozoa

Gondar – June 2015



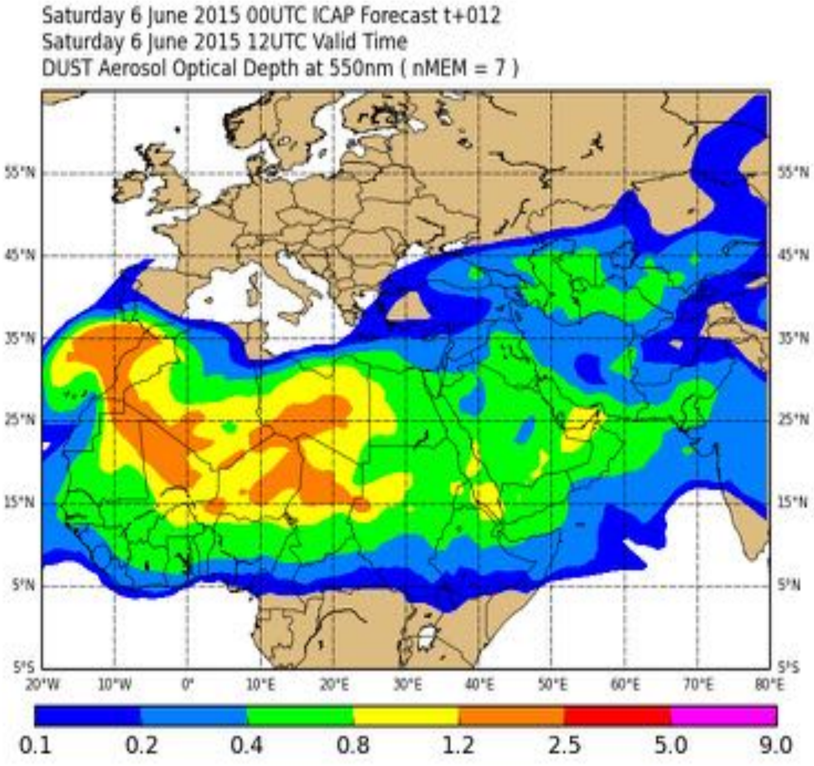


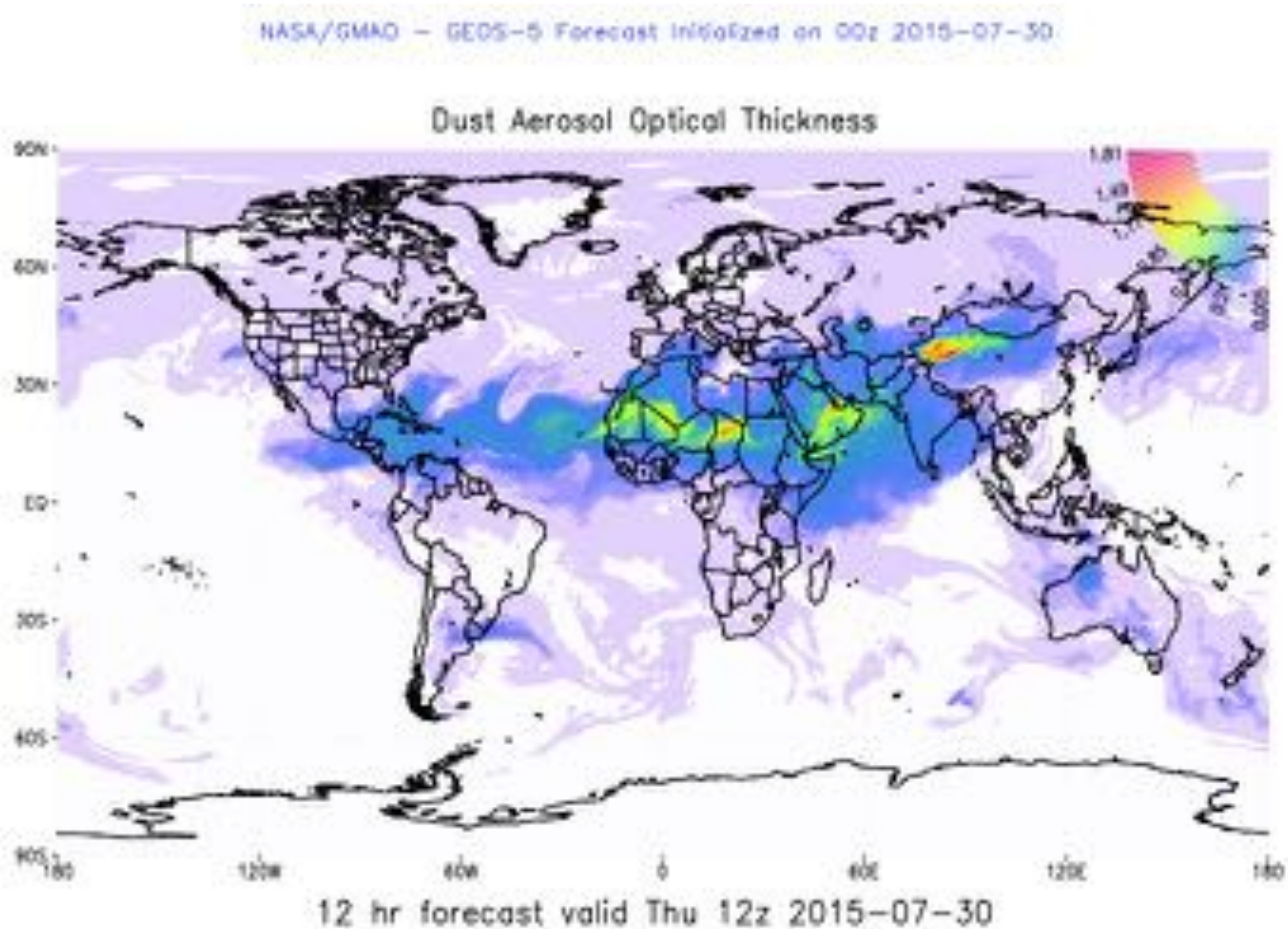




Aerobiological Dynamics During the Monsoon Transition in Northern Ethiopia in an Open-Air Hospital

2015 Air Mass and Culturing Analysis**




NASA/GMAO - GEOS-5 Forecast initialized on 00z 2015-07-30
Dust Aerosol Optical Thickness
90N
60N
30N
EQ
30S
60S
90S
180
120W
60W
0
60E
120E
180
12 hr forecast valid Thu 12z 2015-07-30

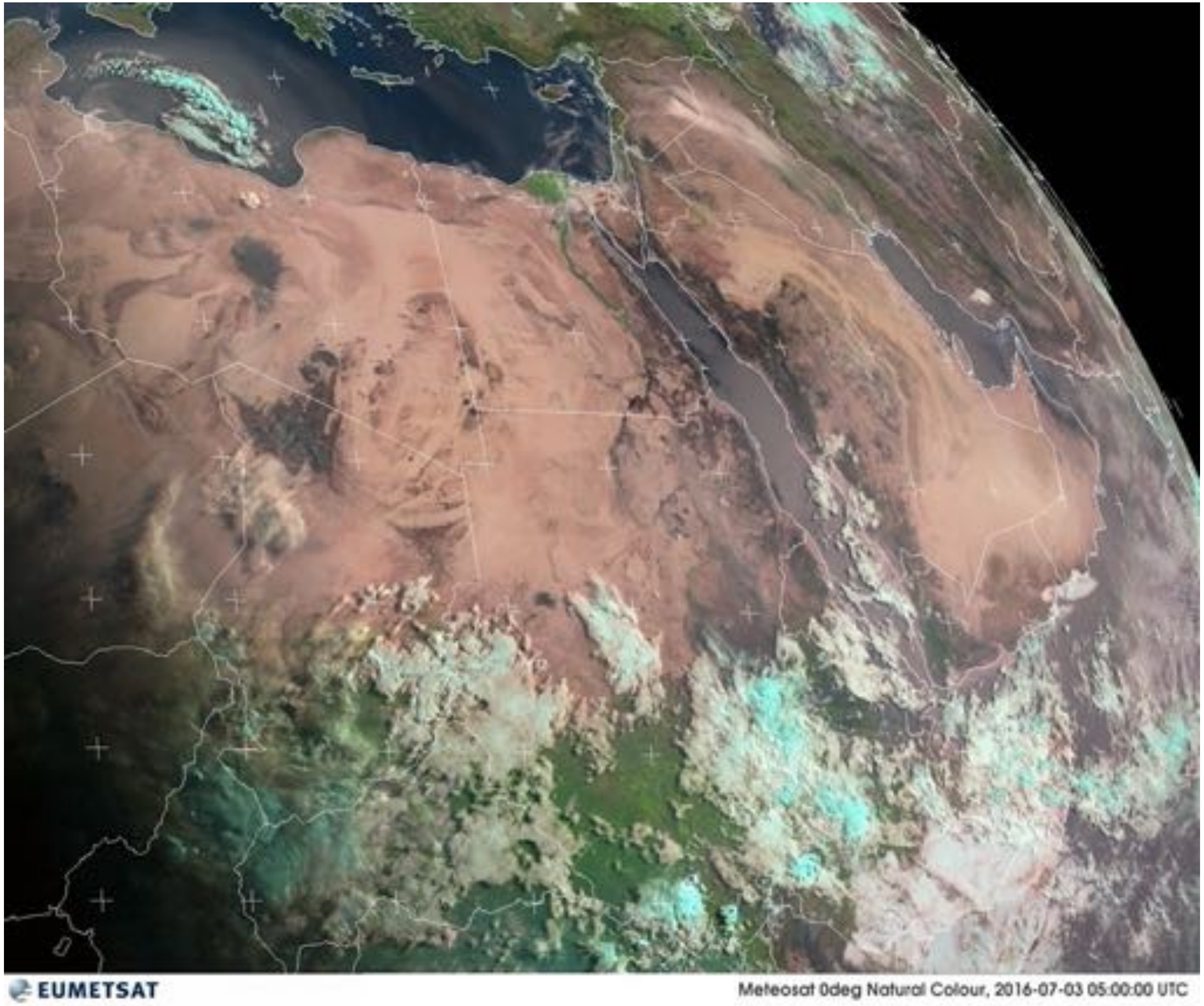
Aerosol Distributions
Preliminary Results 2014 – 2015

2014

- 30 air filter samples for mixed microbiological and chemical analysis
- The number of cultures decreased with decreasing aerosol size (7:1 ratio between the 2.1 mm and 1.1 mm stages and no cultures observed on the 0.65 mm filter)
- Black carbon content appeared to be anti-correlated with microbial population

2015

- Ambient PM2.5 ~0.4μg/m^3
- Ambient PM1 strongly correlated to traffic
- 49 Air filter samples for mixed microbiological and chemical analysis
- Fog and rain days experienced ~30% less PM mass loading
- Large-scale air mass transport can increase PM mass loading by 100%


EUMETSAT
Meteosat 0deg Natural Colour, 2016-07-03 05:00:00 UTC

Results 2016

	Wet Season	Dry Season
Street Level	30% reduction in PM2.5 after significant rainfall events but no statistical difference in microbiology	Factor if 2-3 x higher PM levels relative to wet season
TB Ward	No statistical difference in PM (seasons)	
ICU Ward Leishmaniasis Ward	PM correlated with greater species diversity	Factor of 2-3x higher PM levels relative to wet season
Mortality	30% higher mortality rate in ICU	

*Detailed microbiology data is under review by UG Hospital staff

Summary and Relevance

- The Ethiopian Northern Highlands are a unique transitional region where climate variability, land use change, neglected infectious disease, and rapid socio-political change are simultaneous factors in everyday life
- Aerobiology is a high-impact aspect to be included in future air quality studies
- Air quality risks associated with the combined and effects of food security, public health, resource management, and conflict can only be addressed with comprehensive and integrated social and scientific approaches
- Next steps should include integration of risk perception and response into the study

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  - the National Institute on Minority Health and Health Disparities of the National Institutes of Health under Award Number **U54MD008149**.

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Questions??

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