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Bacillus anthracis in American Soils: From Sample Collection to Data Application

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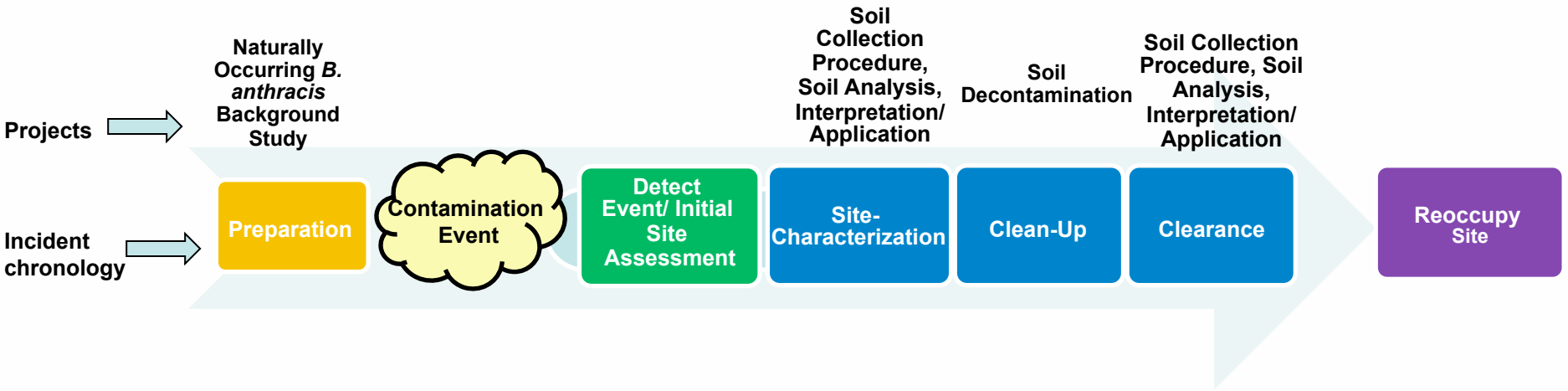
Outline

- Background
- Naturally occurring *Bacillus anthracis (B. anthracis)* study
- Optimization of recovering *B. anthracis* spores from soil
- Sample collection protocol
- Evaluation of soil decontamination technologies
- Data interpretation and application efforts



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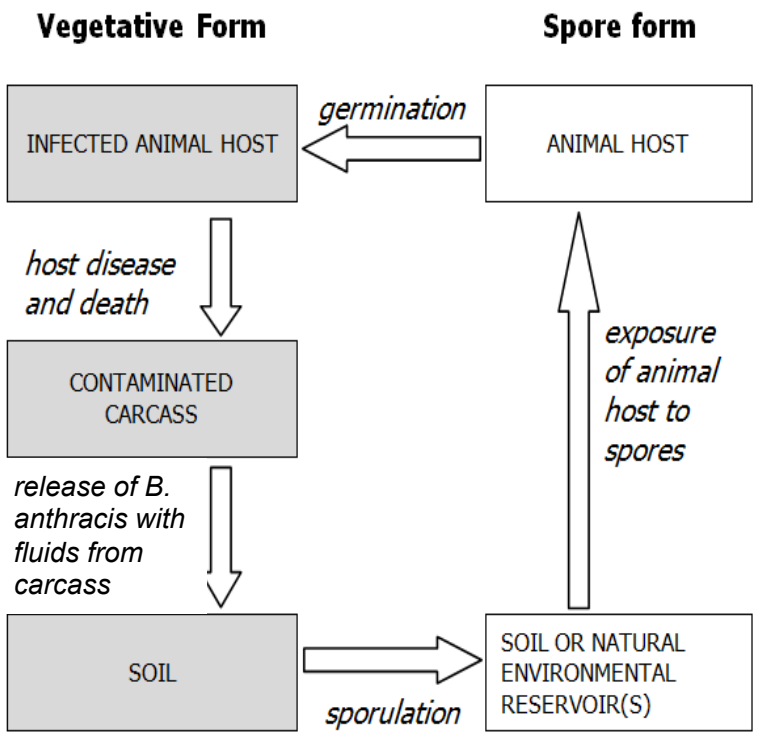
Background

Bacillus anthracis Spores in Soil

- *B. anthracis,* the etiologic agent of anthrax, is naturally occurring in many soil environments and can persist in soil for many years
- Outbreaks of anthrax in wildlife and livestock are often associated with old graves of animals that have died from anthrax and suitable soil conditions
- The presence of *B. anthracis* spores in the environment depends on many factors such as soil type, environmental conditions, ecology, bacterial lifecycle, and persistence



***B. anthracis* natural lifecycle; modified from Schuch and Fischetti (2009).**

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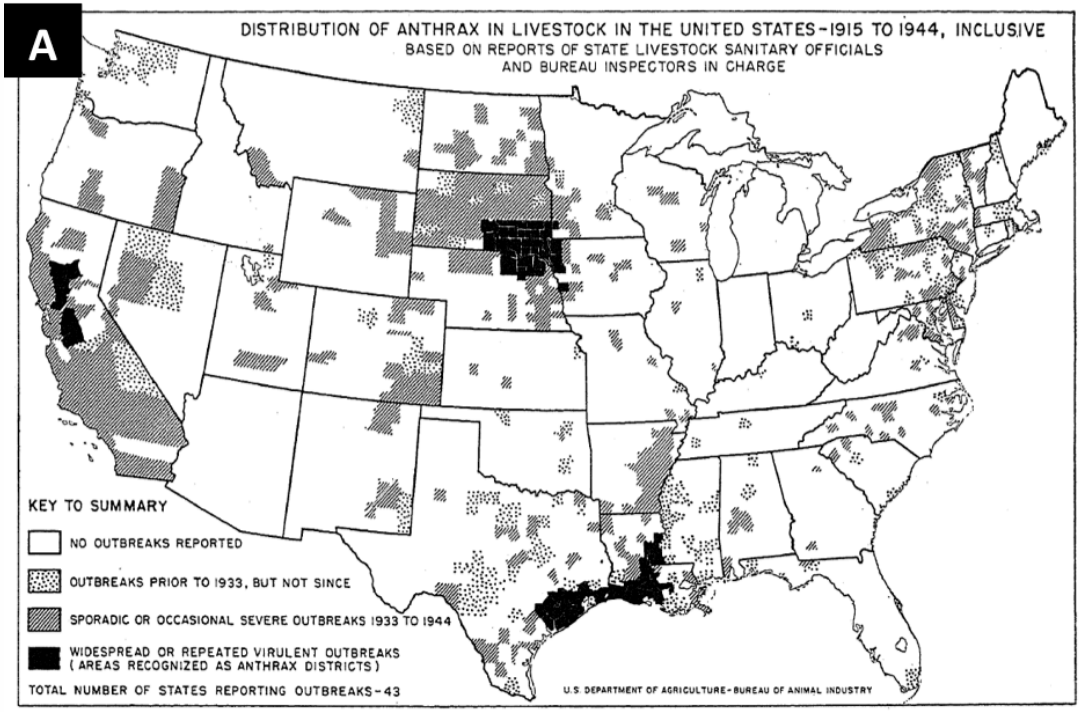
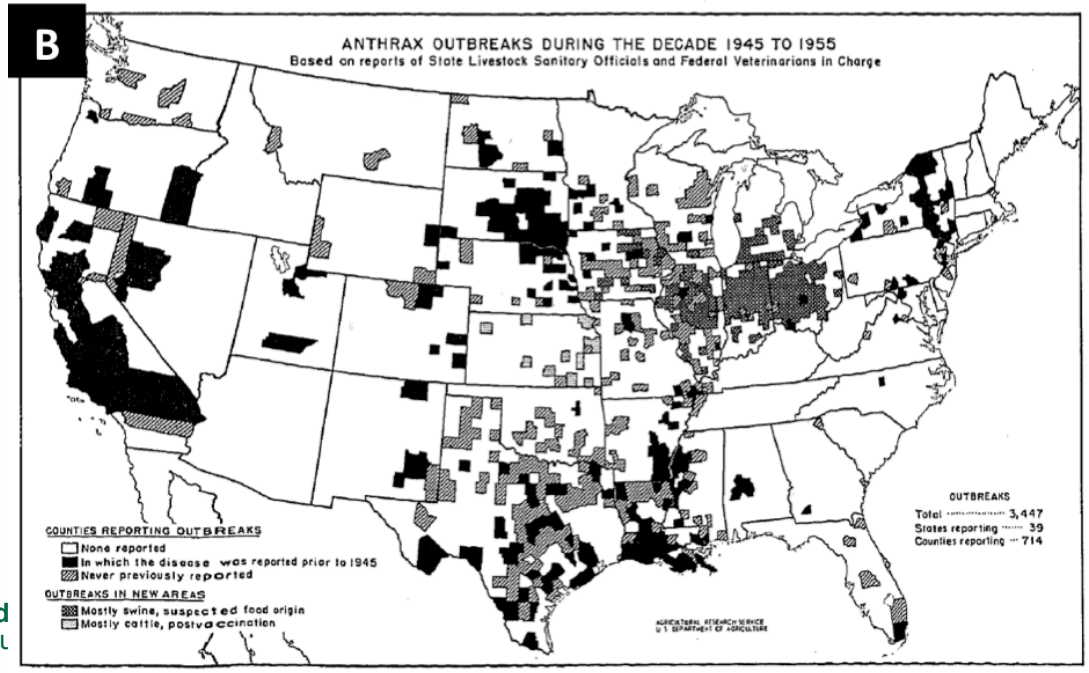
Panel A. Outbreak data for 1915 – 1944 (page 348 – Stein, C.D., 1945. The history and distribution of anthrax in livestock in the United States. Veterinary Medicine. 40(10): 340-349).

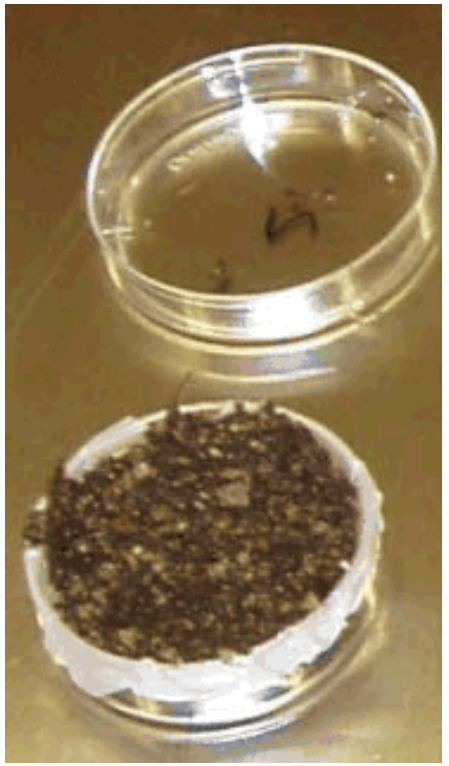


Panel B. Outbreak data for 1945 – 1955. (page 585. Stein, C.D. and B.G. Van Ness. 1955. A ten year survey of anthrax in livestock with special reference to outbreaks in 1954. Veterinary Medicine 50:579-588.



Background of the Problem

- Remediation efforts could be extensive following an aerosol release of *B. anthracis* spores over a wide area
  - Many types of materials and environments may need to be sampled, analyzed, and decontaminated
- Soil remains one of the most difficult sample materials to analyze and decontaminate for *B. anthracis* spores
- Knowledge of *B. anthracis* spore natural occurrence in the environment helps decision makers better prepare for an incident and is a valuable tool for post-event investigations



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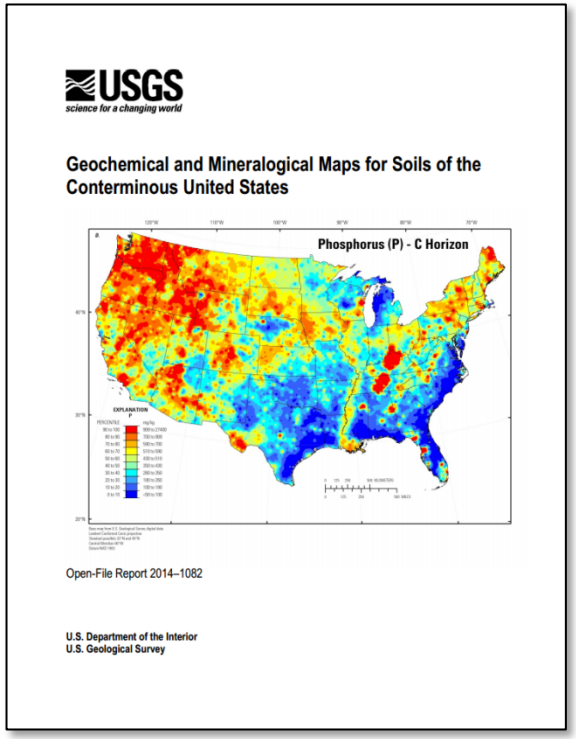
Naturally Occurring *B. anthracis*

U.S. Geological Survey (USGS) North American Soil Geochemical Landscapes Project (NASGLP)

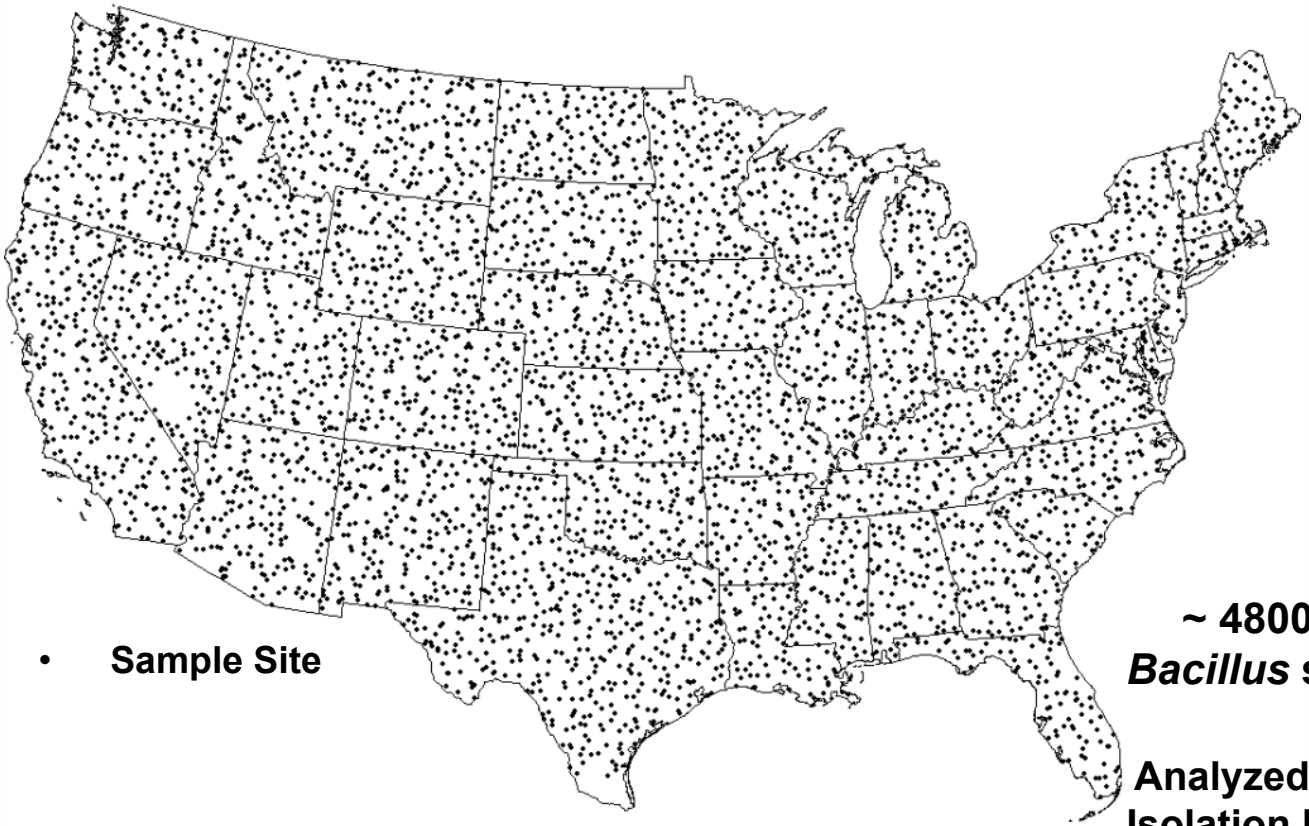
- Soil samples collected at a density of 1 site per 1600 km^2 (~13,500 sites) to expand baseline geochemical and microbiology data for the U.S., Canada, and Mexico.
- Pilot studies began in 2004 and sample collection ran from 2007-2010



http://pubs.usgs.gov/of/2014/1082/

USGS and EPA Sample Analysis

USGS and EPA co-funded an effort to analyze samples from 48 contiguous states for presence of biological agents of interest



- **Sample Site**

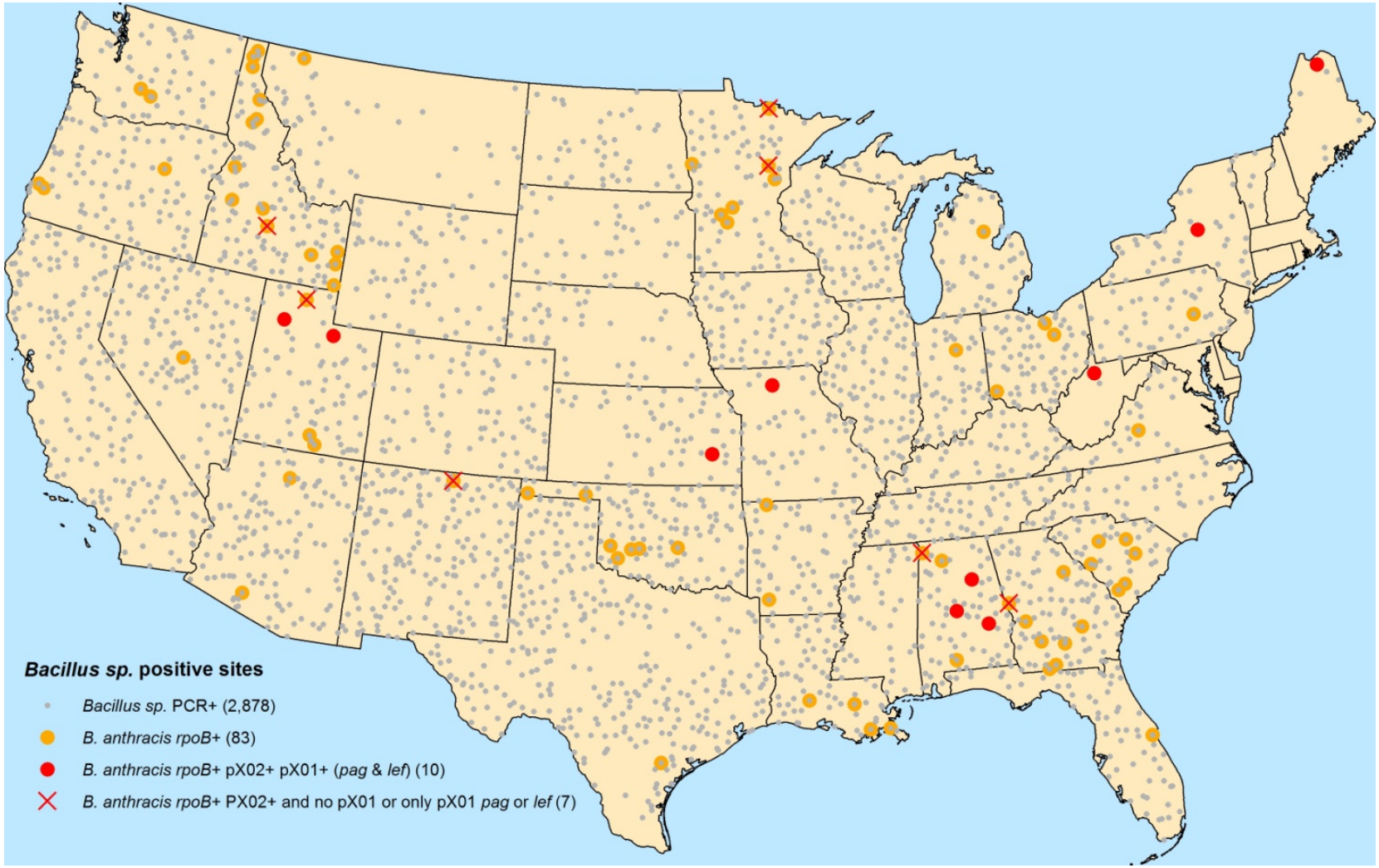
~ 4800 samples analyzed for *Bacillus* species and *B. anthracis*

Analyzed via MoBio Powersoil DNA Isolation Kit and gel electrophoresis to analyze PCR products

Results for *Bacillus* spp. and *B. anthracis* Analysis

Bacillus spp. were detected in 60.3% of the samples and 43 of 48 states

B. anthracis presumptively identified in 83 samples



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Comparison of *Bacillus* Data to Geochemical Data and Outbreak Data

- Determine relationships between the presence of *Bacillus* spp. and *B. anthracis* spores in soils and the following variables:
  - Geochemical data for over 40 major and trace elements
  - Animal anthrax outbreaks (since 2000) vs. geochemical data
- Negative and positive correlations noted for the *Bacillus spp*. data and various elements
- No significant relationships were noted for the *B. anthracis* spore data

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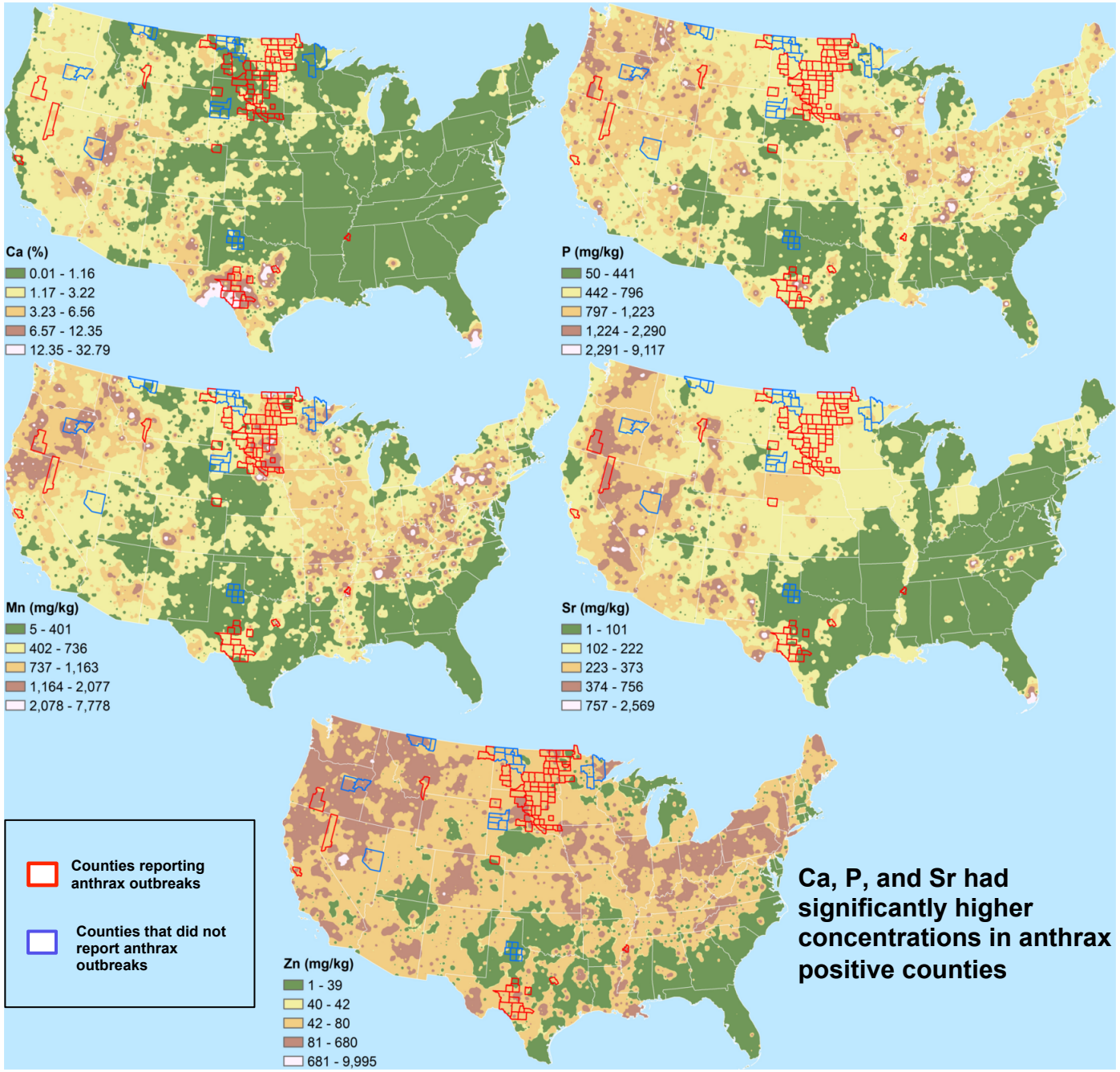
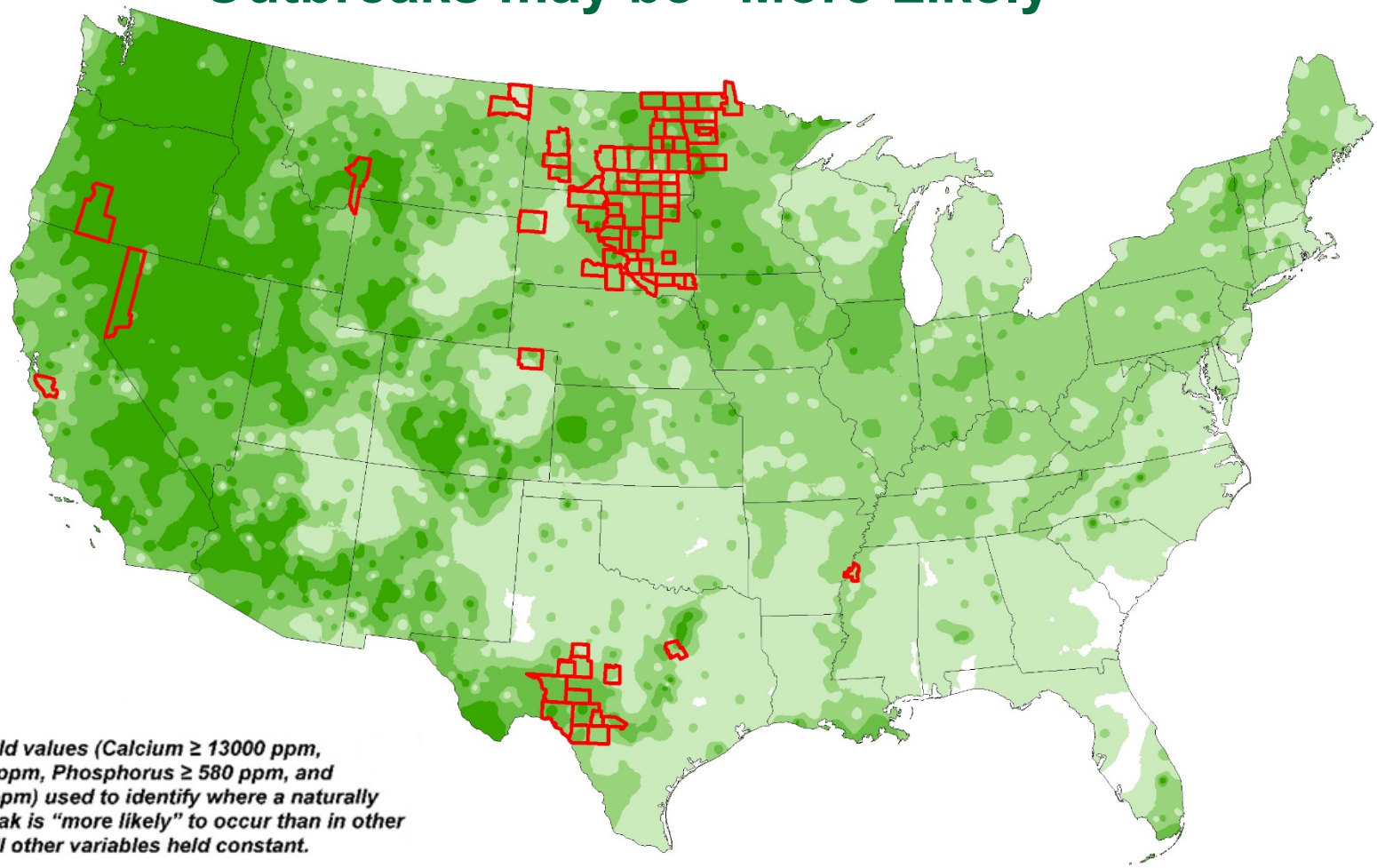
Elemental Data Compared to Counties Reporting/Not Reporting Animal Anthrax Cases



Ca, P, and Sr had significantly higher concentrations in anthrax positive counties

Elemental Thresholds vs. Areas Where Outbreaks may be "More Likely"



Tentative threshold values (Calcium ≥ 13000 ppm, Maganese ≥ 463 ppm, Phosphorus ≥ 580 ppm, and Strontium ≥ 170 ppm) used to identify where a naturally occurring outbreak is "more likely" to occur than in other locations, with all other variables held constant.

Counties

- Counties with Confirmed Agricultural/Wildlife Anthrax Cases since 2000 (78)
- No Thresholds Met
- One Threshold Met
- Two Thresholds Met
- Three Thresholds Met
- All Thresholds Met

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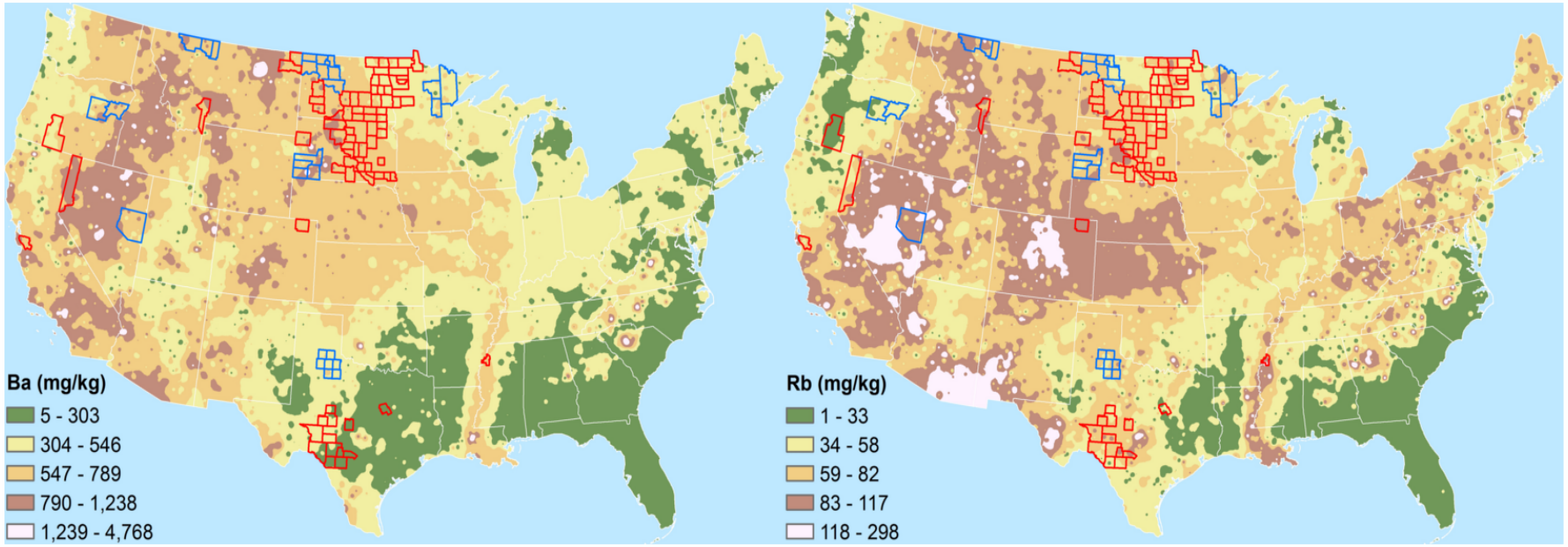
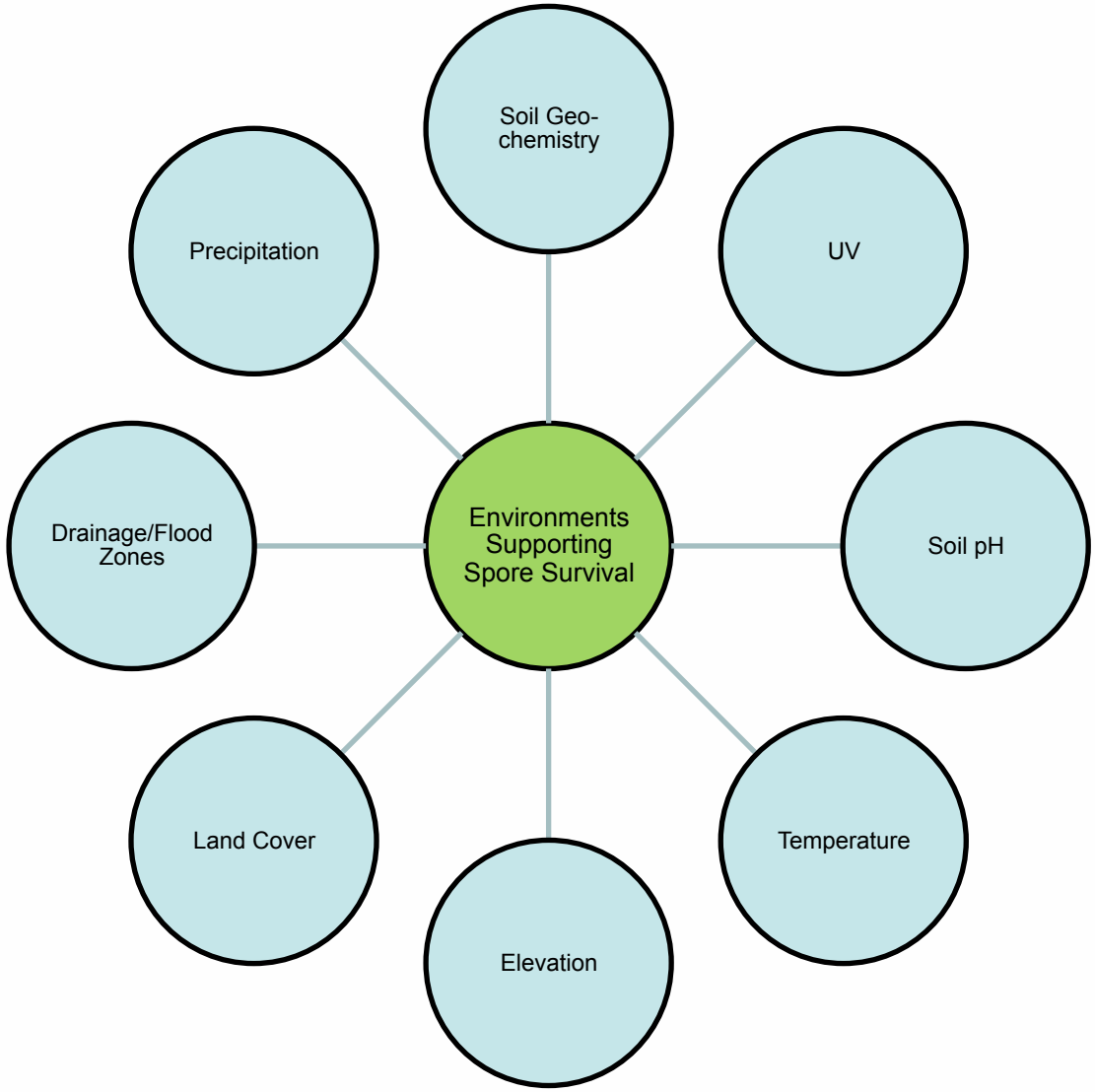
Elements Present in Anthrax Negative Counties



Additional Variables Being Considered

Spatially determine spore survival using these variables



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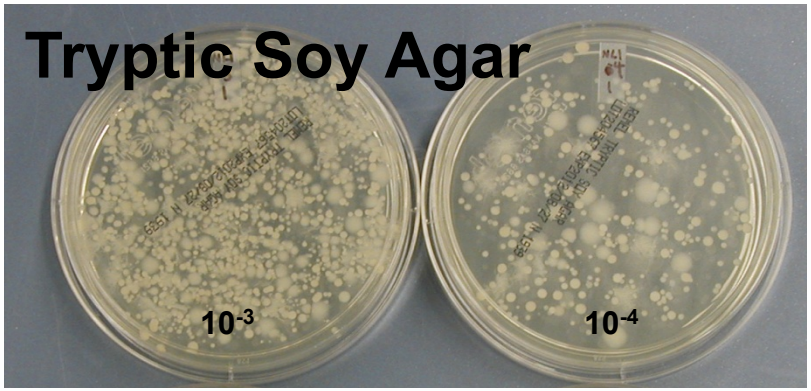
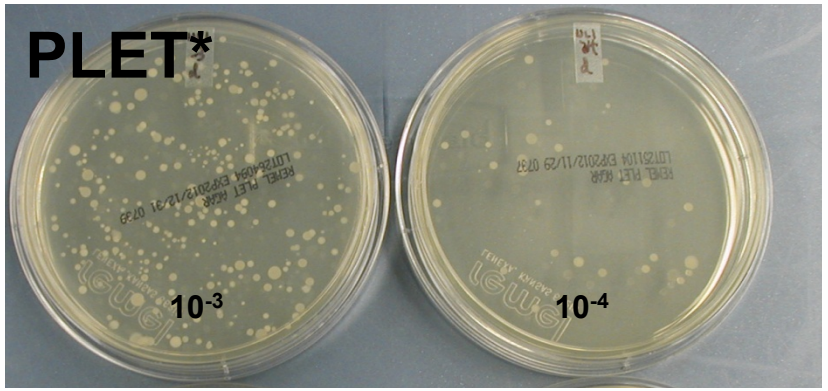
Optimization of Recovering *B. anthracis* Spores from Soil

Need for a Standardized Soil Method

- A standardized method for recovery of *B. anthracis* spores from soil is needed
- Detection of *B. anthracis* spores in soil is challenging due to interferences, inhibitors, impurities, and other organisms in the soil that may reduce recovery efficiency

Native (nonsterile) Agvise Loam Soil with no *B. anthracis* spores added shows growth in non-selective and selective media of background organisms

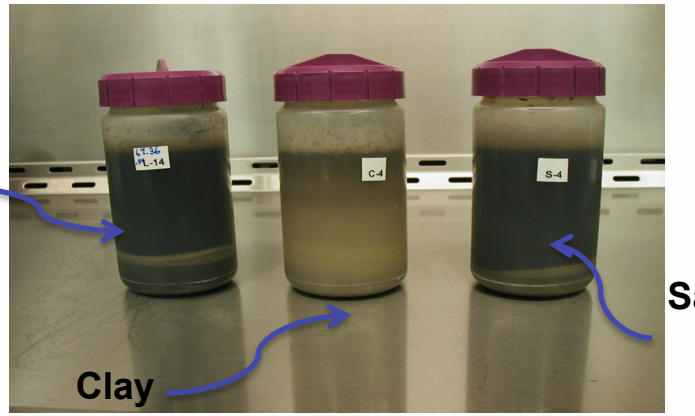




* *polymyxin-lysozyme-EDTA-thallous* acetate

Development and Verification of an Optimized Extraction Method

- USGS and EPA project team working on optimizing extraction of *B. anthracis* spores from soil (processing step prior to DNA extraction and further analysis)
- The method is being developed and verified using three soil types and two sample sizes (9 g and 45 g)
- Method consists of a series of washes and centrifugation steps to concentrate the spores into a pellet
- Method has been verified in loamy and sandy soil



Lessons Learned for Method to Recover and Analyze for *B. anthracis*

- A larger sample size increases the probability that *B. anthracis* spores at a site will be included in samples to be processed
- Background organisms and other interferences makes analysis of soil samples using culture difficult
  - Currently have to use MoBio PowerSoil DNA isolation kit and qPCR for analysis
- Re-analysis of USGS soil samples using improved method may help identify additional locations where *B. anthracis* may be present/found

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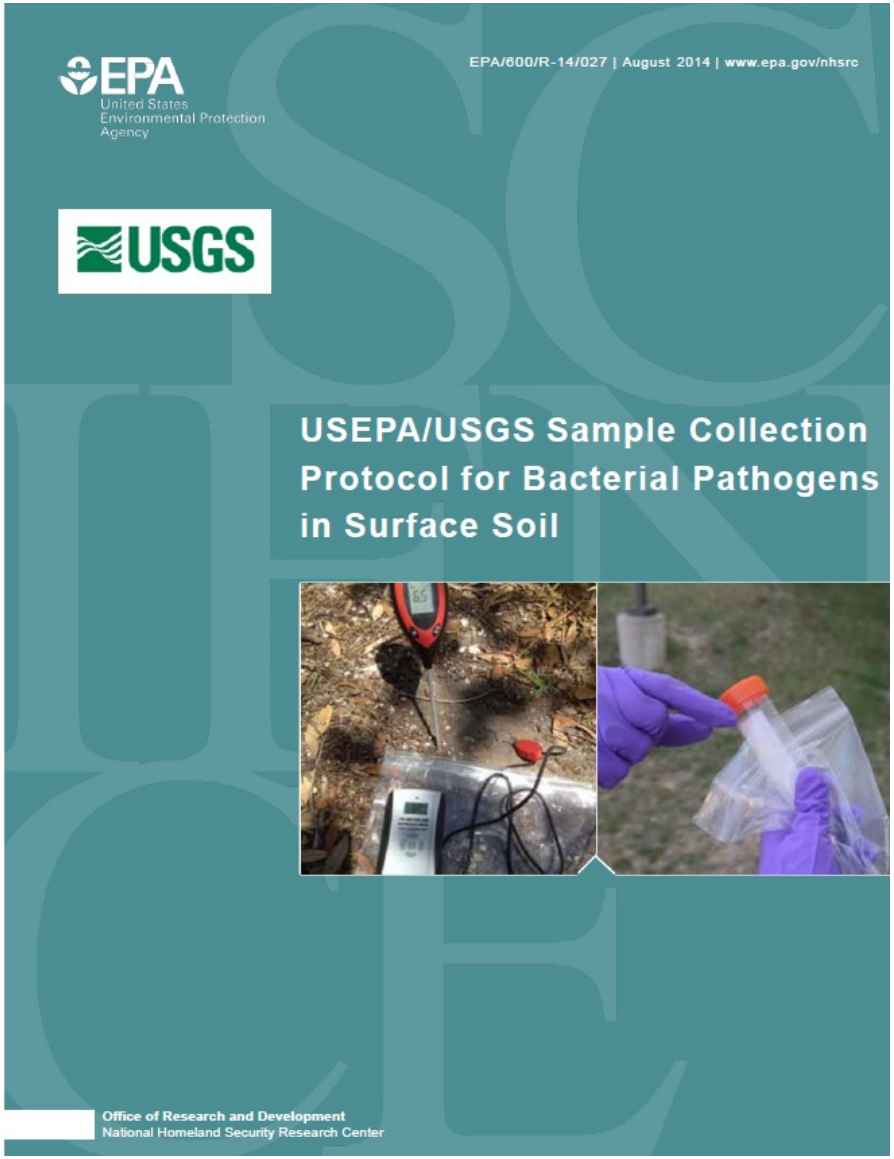
Soil Sample Collection Protocol

EPA/USGS Sample Collection Protocol for Bacterial Pathogens in Surface Soil

- Protocol for collecting, handling, and shipping of soil samples for detection of naturally occurring pathogens of concern

- Based on the procedures used by U.S. Geological Survey (USGS) during its North American Geochemical Landscapes Pilot Studies



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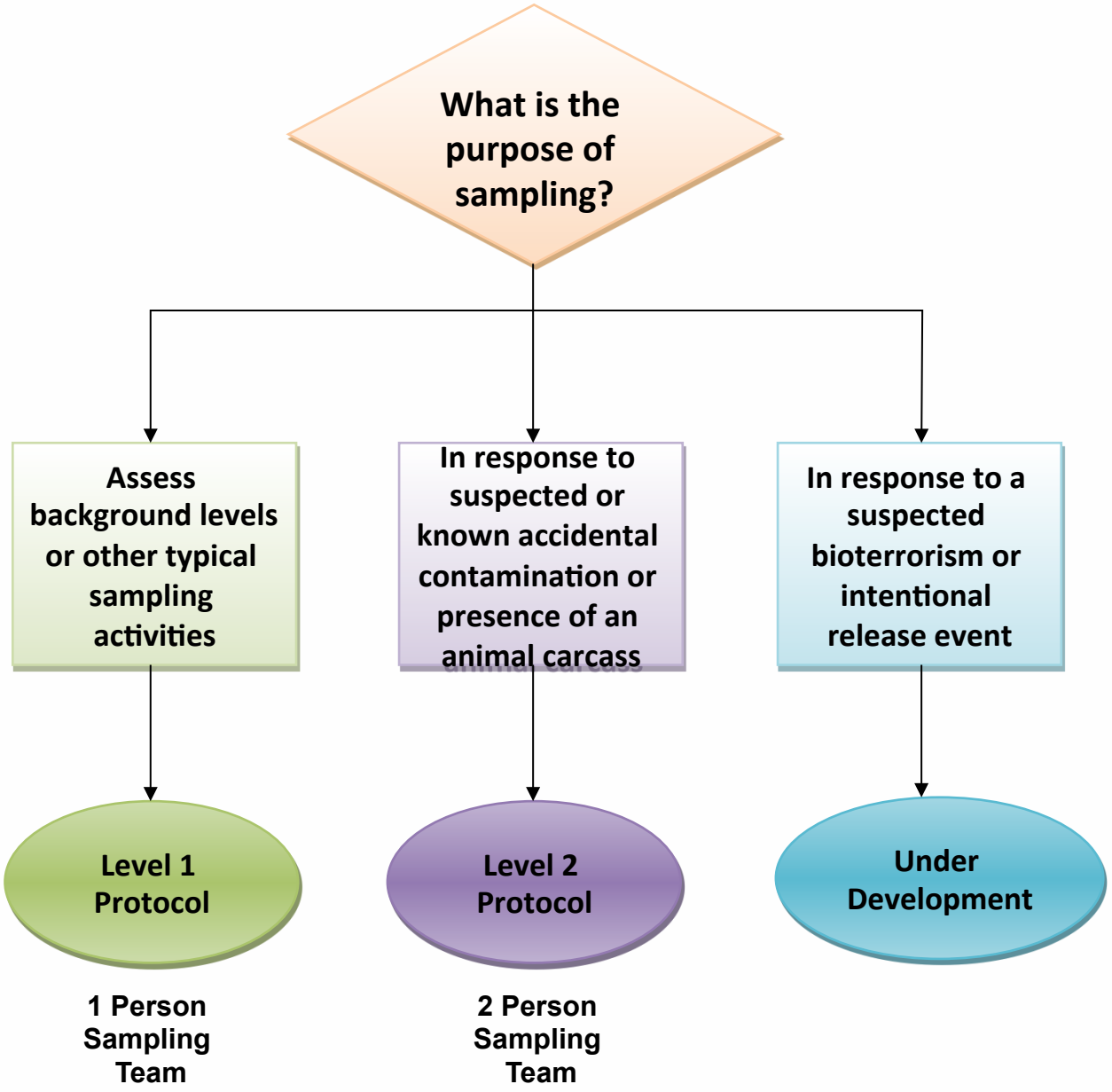
Sample Collection Protocol Used For:

1. **Surveillance to determine naturally occurring background levels in soil (i.e. no suspicion of contamination)**

2. **For suspected or known accidental contamination (i.e. presence of animal carcass)**



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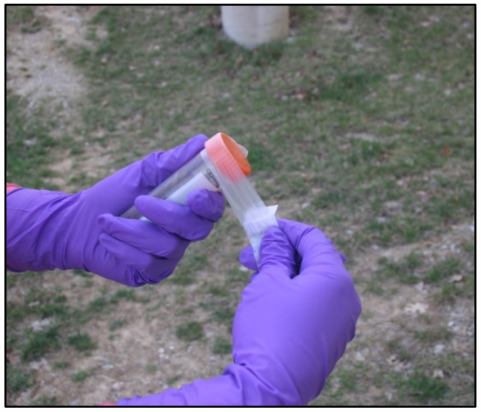
Protocol Overview

- 50 mL sterilized tubes
- Applicable for most types of soil
- Top 0-5 cm of soil
- Step-by-step instructions
- Soil moisture, temperature, pH, GPS location, and other landscape characteristics recorded
- Includes QA Samples
- Field log and chain of custody forms provided



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Soil Decontamination

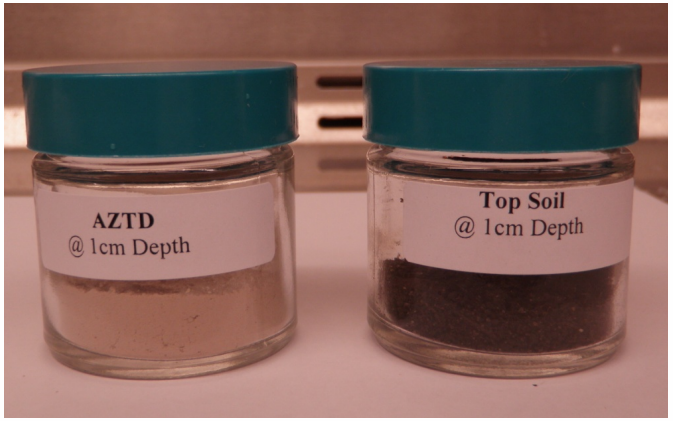
Soil Decontamination Technologies Evaluated

- Chlorine dioxide (ClO_2) gas
- Aqueous ClO_2 solution
- pH-amended (acidified) bleach
- Sodium persulfate activated with hydrogen peroxide
- Methyl bromide
- Metam sodium
- CASCAD
- Oxonia Active (peracetic acid)
- Chloropicrin
- Natural attenuation of vegetative *B.a.**

*Spores were purposefully germinated into cells in this scenario to see how long they would last

Testing Parameters

- Tests were conducted to assess decontamination efficacy
- *B. anthracis* (Ames strain) and *B. subtilis* (ATCC 19659)
- Topsoil and Arizona Test Dust (AZTD) among soil types used
- Other variables tested depended on decon tech., but included:
  - contact time
  - number of applications (liquids)
  - decontaminant concentration
  - temperature, relative humidity (RH)
  - soil depth
  - soil moisture



Inoculated at ~1x10^8 CFU of viable spores using 100 µL liquid suspension

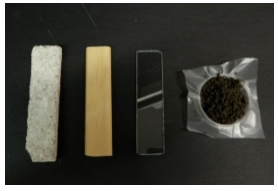
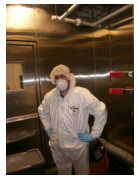
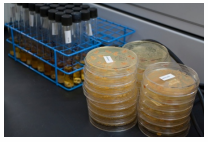
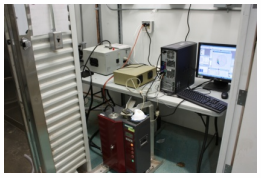
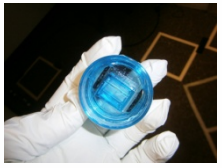
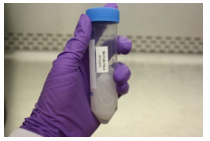
Lessons Learned for Soil Decon

- Decon efficacy: > 6 log reduction for *B. anthracis* spores obtained on both soil types for ClO_2 gas, sodium persulfate, methyl bromide, and metam sodium

- Persistence of vegetative *B. anthracis* in topsoil, at ambient lab temperature and RH (natural attenuation), was 5 days

- Soil Type: AZTD generally easier to decon, but depends on decontaminant (e.g. sodium persulfate efficacy for *B. anthracis* similar for both soil types)

- Soil depth: In tests with ClO_2 gas, increasing soil depth significantly impacted efficacy. Further research needed to asses impact of soil depth


Soil Decon References

EPA/600/R-12/517

EPA/600/R-12/516

EPA/600/R-13/110

EPA/600/R-010/080

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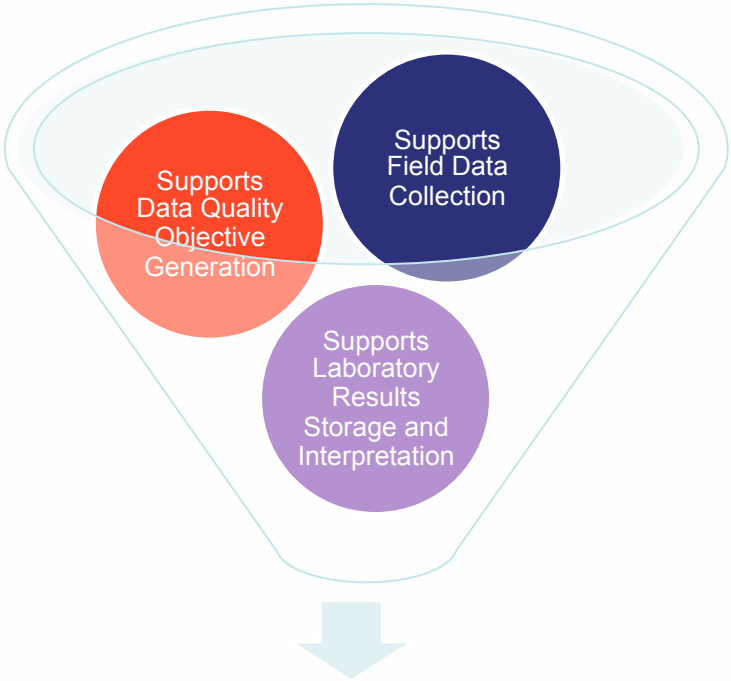
Data Interpretation and Application

Ongoing Efforts in Data Usability and Data Management

- Effort to determine microbial data usability including:
  - Identifying data quality objectives for microbial agents
  - Determining the state of the science on appropriate use of microbial data collected in the field and subsequently analyzed in the laboratory
- Evaluation of existing data management tools for data collection and analysis



Questions?

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