

# Peer Review Summary Document

(2/18/2014)

[http://www.usgs.gov/peer\\_review/docs/anthrax\\_geochemistry-soils\\_contiguousUS.pdf](http://www.usgs.gov/peer_review/docs/anthrax_geochemistry-soils_contiguousUS.pdf). [20 KB PDF]

## Title and Authorship of Information Product Disseminated

Anthrax and the Geochemistry of Soils in the Contiguous United States, By Griffin, D.W., E. Silvestri, C.Y. Bowling, T. Boe, D.B. Smith and T.L. Nichols.

## Peer Reviewers Expertise and Credentials

Peer Reviewer #1: USGS research microbiologist with expertise in environmental microbiology, public health microbiology, and biostatistics.

Peer Reviewer #2: Senior USGS research geologist with expertise in carbonate environments.

Peer Reviewer #3: Various U.S. Environmental Protection Agency (EPA) National Homeland Security Research Center reviewers.

## Charge Submitted to Peer Reviewers

USGS reviewers were asked to make an objective evaluation of the research.

## Summary of Peer Reviewer Comments and USGS Response to Peer Reviewers Comments

USGS responses to specific comments from the peer reviewers are in italics below.

### Peer Reviewer #1

The reviewer provided constructive editing of the document and suggested changes that strengthened the manuscript. A summary of the USGS responses to the reviewer's comments are in italics as follows.

- *All of the reviewer's editorial edits were accepted.*
- Comment: You've capitalized "State" several times throughout the manuscript when used as a general term or modifier. I think it should only be capitalized when used part of a state's name. But I could be wrong, so check on this.
  - *Changed all to lower case where not used with state name*
- Comment 2. topological?
  - *Changed term from "topical?" to "topological"*
- Comment 3. By definition "alkaline" is a pH value greater than 7.0. Consider just leaving out "...(alkaline)...".
  - *Deleted alkaline*

- Comment 4. Consider rephrasing to something like “...Data from these sample sites were either combined into a single data set or sorted by state. Each data set was then evaluated for significance differences between counties with and without confirmed anthrax outbreaks (89 sample sites located within 27 counties). ...”
  - *Adopted the suggested sentence format*
- Comment 5. I must be misinterpreting this sentence and the paragraphs below this one. There are no negative values in Table 1 and p-values are never negative as they represent a probability and you can’t have a negative probability. What are “negative” and “positive” differences referring to? I’m reading it like they are referring to one of your statistical tests and up to this point in the paper you’ve just used the p-values to say there are significant differences between an element’s concentrations in an anthrax(+) vs. anthrax(-) sample site.
  - *Added (meaning the concentration was higher in anthrax counties) after the word positive and (concentration was lower in anthrax counties).*

**Peer Reviewer #2**

The reviewer provided a thorough edit of the document along with suggested changes in format to aid in interpretation. A summary of the USGS responses to the reviewer’s comments are in italics as follows:

- *All editorial style corrections were accepted and utilized throughout the document.*
- Page 2, comment 2. Spell in full at beginning of sentence.
  - *Change made.*
- Page 3, comment 3, Page 5, comment 5, and Page 7, comment 7. The states were abbreviated above. Suggest consistency (spelled out or abbreviated) whichever way you choose.
  - *As suggested by Reviewer 3, full names were used until all the states where mentioned and then after the methods section, abbreviations were used consistently throughout the rest of the document.*
- Page 4, comment 4. Sites are not being collected.
  - *Accepted suggested change.*
- Page 5, comment 6. Are these numbers zip codes? Please clarify.
  - *No these are sample site numbers and were removed from the document and placed in supplemental table #1.*
- Page 8, comment 8. This makes no sense. Please reword and use ‘observed’ only once.
  - *This whole section was reformatted for clarity.*

**Peer Reviewer #3**

Collectively the EPA reviewers (identified in this document as Reviewer #3) provided constructive edits and changes to the documents structure. The reviewer comments and USGS responses to them are in the table below.

| <b>Section</b> | <b>Comment</b>                                                                                                                                       | <b>Response</b>                                                                                              |
|----------------|------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------|
| <i>General</i> | <i>Suggest spelling out elements and states on first usage then use their abbreviations after that. I made a few edits but this inconsistency is</i> | <i>Started following their first use in methods section since they were not mentioned together or at all</i> |

|                |                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
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|                | <i>pretty pervasive throughout the manuscript</i>                                                                                                                                                                                                                                                                                                                                                                  | <i>prior to that.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |
| <i>General</i> | <i>I made edits to put manuscript into passive case (e.g. This was done) instead of active case (e.g. we did this).</i>                                                                                                                                                                                                                                                                                            | <i>Accepted all suggested edits.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <i>General</i> | <i>Page 6, line 132- you used the detection limit as the concentration for elements present below the detection limit. How the elements that are present below detection limits are numerically handled may or may not have profound effect on the analysis. Do you see if the statistical analysis results changed by setting the concentration of elements that were there at &lt; DL to Zero instead of DL?</i> | <p><i>Six elements had &lt; values, S had 2 out of the 120 positive county sites, none in the negative counties so this wouldn't make a significant difference. The other Cs, Ag, Cd, Te and Se had numerous. Only Cs gave a sign diff but 106 of the 120 positive county sites were less than values and in negative counties it was 64 out of the 89 sites. Added the following text:</i></p> <p><i>Methods</i><br/> <i>In the USGS Geochemical Landscape Project element concentration data set there are values expressed as below minimum detection limits (MDL) for certain elements (Ag = 189 of 209, Cs = 170/209, Cd= 19/209, S = 2/209, Se 67/209 and Te 198/209 data points). For statistical analyses those values were set at the MDL for the respective elements (e.g. &lt;1 is set at 1).</i></p> <p><i>Results</i><br/> <i>Cesium data produced a significant P value below 0.05 but this was dismissed due to the fact that 170 of the 209 data points were below the MDL. Of the remaining four elements (Ag, Cd, Te and Se) with MDL data none produces P-values below 0.05.</i></p> |
| <i>General</i> | <i>References should be at the end of the text, with figures and tables afterwards. Either that or embed the figures and tables into the text</i>                                                                                                                                                                                                                                                                  | <i>Yes, when formatted for submission these will be moved to the proper location. They were put</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |

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|                           | <i>at the right location. Whatever the journal wants.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | <i>them here for ease of internal review.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <i>General</i>            | <i>Text on some of the supplemental figures is too small to read</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | <i>Increased font size to make more legible.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <i>General</i>            | <i>Table 1 is really hard to understand. Could you maybe use some color coding to emphasize which elements were statistically significant? Cells could be shaded white, yellow, green for example.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               | <i>Modified the title to help clarify what was being presented: 'The significance (Mann-Whitney U test P-values – in bold where &lt; 0.05) of elemental concentrations (averages in brackets [#/#] where an overall or greater than two multi-state significant P-values) in counties reporting outbreaks or cases of anthrax in livestock and wildlife versus counties that have not reported outbreaks or cases of anthrax since the year 2000.'</i>                                                                                                                                                                                                                                                                                                                                     |
| <i>General Statistics</i> | <p><i>The manuscript states : The non-parametric Mann-Whitney U test was utilized to evaluate differences in geochemistry between counties where outbreaks or cases had been reported since the year 2000 and counties within the same states where no cases were noted for the same time period using SPSS (IBM, Tampa, FL) (Dytham 1999). In the USGS Geochemical Landscape Project element concentration data set there are values expressed as below minimum detection concentrations for certain elements. For statistical analyses those values were set at the minimum detection concentration for the respective elements (e.g. &lt;1 is set at 1).</i></p> <p><b>Comments:</b></p> <p><i>What % of the data is below minimum detection limit (for each county)? If it is relatively high, assuming a value of 1 will bias the conclusions. % of below minimum detection limit should be provided in the manuscript.</i></p> | <p><i>Added that data ...in methods... In the USGS Geochemical Landscape Project element concentration data set there are values expressed as below minimum detection limits (MDL) for certain elements (Ag = 189 of 209, Cs = 170/209, Cd= 19/209, S = 2/209, Se 67/209 and Te = 198/209 data points). For statistical analyses those values were set at the MDL for the respective elements (e.g. &lt;1 is set at 1). ...in results... Cesium data produced a significant P-value below 0.05 but this was dismissed due to the fact that 170 of the 209 data points were below the MDL. Of the remaining four elements (Ag, Cd, Te and Se) with MDL data none produced P-values below 0.05.</i></p> <p><i>2. Did not do covariant analyses as there are other influences such as</i></p> |

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|                       | <p><i>How about performing a logistic regression (response Y is positive or negative for anthrax, covariate X (or multiple X's) is the element concentration (or multiple element concentrations)? &lt;1 element concentration should be treated as censored data. There are software programs for censored data.</i></p> <p><i>I think, the above <b>comments should be addressed</b> before submitting to a journal.</i></p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | <p><i>precipitation and temperature related to outbreaks and cases....that data is not available at this time and thus absence would confound output interpretation...the approach here was more direct and focused...Can we identify individual elements concentrations that appear higher or lower in anthrax positive and anthrax negative counties...and could we ID potentially important concentrations levels.</i></p> |
| <p><i>General</i></p> | <p><i>This paper describes an observational study in which the concentration of several elements in soil were measured. The results from soils from counties with anthrax outbreaks or events were compared with soil from counties that did not have an anthrax outbreak. The comparison suggested that higher concentrations of Ca, Mn, P and Sr are associated with outbreaks. The authors propose to use this data to set concentration thresholds which would indicate whether the probability of a future anthrax event was "potential" or "probable".</i></p> <p><i>Overall, I thought this was a good study- I did have several editorial comments that are included in the traced changes version of this paper. My only concern was the establishment of thresholds and what they mean. For instance, when the authors set a threshold by saying the likelihood of an outbreak is "probable" what does that translate to- is the likelihood of an event greater than 1%? 10%? 50%? Also, since this is only observational data thus far, would these be "tentative" thresholds, at least until more observation and testing is done?</i></p> | <p><i>Added tentative where suggested and in the abstract at the first mention of threshold...Did not change it in sentence where the threshold values were called preliminary as that would be redundant...Also changed probable to likely throughout the text.</i></p>                                                                                                                                                      |

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| Page 1   | "Anthrax and the <del>geochemistry</del><br><del>Geochemistry</del> of <del>soils</del> Soils in the<br><del>contiguous</del> Contiguous United<br>States"                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | Accepted.                                                                                                             |
| Page 2   | "Statistically significant threshold<br>values of the lowest concentrations<br>of each of these elements (Ca =<br>0.43 wt. %, Mn = 142 mg/kg, P =<br>180 mg/kg and Sr = 51 mg/kg)<br>and significant average<br>concentrations (Ca = 1.3 wt. %,<br>Mn = 463 mg/kg, P = 580 mg/kg<br>and Sr = 170 mg/kg) were<br>identified from anthrax-positive<br>counties as prospective<br>investigative tools in determining<br>whether an outbreak was<br>'potential' or 'probable' at any<br>given geographic location in the<br>contiguous United States."<br><br>Delete extra space between 1. And<br>3. Add hyphen between anthrax<br>and positive                                                                  | Accepted.                                                                                                             |
| Page 2   | "Historical data on environmental,<br>weather/climate, and geographical<br>factors that influence the<br>occurrence of these infections are<br>well known and include..."                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Accepted.                                                                                                             |
| Page 2/3 | "Historical data on environmental,<br>weather/climate and geographical<br>factors that influence the<br>occurrence of these infections are<br>well known and include; 1)<br><del>(weather/climate)</del> warm seasons<br>during dry periods that follow<br>moderate to heavy precipitation<br>events <del>(weather/climate)</del> , 2)<br><del>(environmental)</del> regions containing<br>post-flood organic detritus and/or<br>short dry grazing grasses<br><del>(environmental)</del> and 3) <del>(geology)</del><br>topological lows such as<br>waterholes or riverbanks,<br>calcareous and alluvial soils with<br>elevated nutrient content and pH<br>values greater than 6.0<br><del>(geology)</del> ." | Accepted.                                                                                                             |
| Page 3   | "Other geological factors that may<br>influence B. anthracis outbreak<br>occurrence, as noted through in<br>vivo or in vitro observations, are                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | Left this as is as to present<br>that certain elements are<br>known to be needed by this<br>pathogen...thought the in |

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|        | <p>elevated phosphate (results in higher protective antigen production), magnesium, sodium, copper, zinc (needed for lethal factor production) and manganese (typically found in very low concentrations in calcareous soils and needed for gene regulation of exotoxins and antibiotics) (Griffin and others 2009; Hugh-Jones and Blackburn 2009; Kochi and others 1994; Weinberg 1987; Wright and others 1970). "</p> <p>Difficult to read. Maybe break up into a few sentences.</p> | <p>parenthesis points would prevent from having to mention each of those elements again in a following sentence.</p>                                                                         |
| Page 3 | <p>"Two separate groups of <i>B. anthracis</i>, the 'Ames' and western North America (WNA) clades, are responsible for <del>disease anthrax</del> outbreaks in North America."</p>                                                                                                                                                                                                                                                                                                     | Accepted.                                                                                                                                                                                    |
| Page 3 | <p>"Outbreaks of anthrax are a common occurrence in the contiguous United States and they are typically constrained to a few geographical regions (e.g., Texas, Minnesota, Montana and the Dakotas)."</p> <p>Maybe spell states out on first occurrence and abbreviate all other times.</p>                                                                                                                                                                                            | Abbreviated after the start of the methods section where all 7 states are mentioned together...that way could avoid abbreviations for some and not others when they were mentioned together. |
| Page 4 | <p>"Given the geographic restriction of most annually-occurring cases and outbreaks of anthrax in the contiguous United States, <del>we evaluated</del> geochemical data obtained by the U.S. Geological Survey's (USGS) 'North American Soil Geochemical Landscapes Project' <del>were evaluated</del> to determine which elements may influence the background distribution of this pathogen."</p>                                                                                   | Accepted.                                                                                                                                                                                    |
| Page 4 | <p>"This represents a resolution of ~1 site per 1,600 square kilometers and a report of the project and data <del>is are</del> available at <a href="http://pubs.usgs.gov/ds/801/">http://pubs.usgs.gov/ds/801/</a>."</p>                                                                                                                                                                                                                                                              | Accepted.                                                                                                                                                                                    |

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| Page 4                | <p>"Using a <b>Generalized Random Tessellation Stratified Design</b> for sample site selection, 4,857 sites were utilized for sample collection (Smith and others 2013)."</p>                                                                                                                                                                                                                                                                                                                                                                                                                                       | <p>Smith and others 2013 is the citation for the use of this tool.</p> |
| Page 4 and throughout | <p>"In short, the &lt;2-mm fraction of each sample that was collected from a depth of 0 to 5 cm below the soil surface was analyzed for <b>aluminum</b> (Al), arsenic (As), calcium (Ca), iron (Fe), mercury (Hg), potassium (K),....."</p> <p>Use abbreviations after this now that they have been defined.</p>                                                                                                                                                                                                                                                                                                    | <p>Accepted, changed throughout the document.</p>                      |
| Page 4                | <p>"<del>Bacillus-B.</del> anthracis case and outbreak data by State County, 2000 – 2013: Figure 1 illustrates state counties reporting outbreaks or cases <del>and of</del> anthrax since 2000 (red counties)."</p>                                                                                                                                                                                                                                                                                                                                                                                                | <p>Accepted.</p>                                                       |
| Page 5/6              | <p>"The following counties were utilized for statistical evaluation: 1) Minnesota - Clay, Kittson, Lake of the Woods, Marshall Pennington, Polk and Roseau; 2) Montana - Gallatin, Sheridan and Roosevelt; 3) Nevada – Washoe. North Dakota - Barnes, Cass, Grand Forks, Nelson, Pembina, Stark, Steele, and Traill; 4) Oregon – Klamath; 5) South Dakota - Aurora, Brown, Brule, Buffalo, Charles Mix, Corson, Day, Dewey, Hand, Hughes, Hyde, Lyman, Marshall, Mellette, Potter, Spink, Tripp and Walworth; <del>and</del> 6) Texas - Edwards, Irion, Kinney, McCulloch, Real, Sutton, Uvalde and Val Verde."</p> | <p>Accepted.</p>                                                       |
| Page 6                | <p>"In summary, there were 118 sample sites located within 46 counties. Data from these sample sites were either combined into a single data set or sorted by state. Each data set was then evaluated for significant differences between counties with and without (89 sample sites located within 27 counties) confirmed anthrax outbreaks or cases. These anthrax-free counties included: 1)</p>                                                                                                                                                                                                                 | <p>Accepted.</p>                                                       |

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|               | <p>Minnesota – Aitkin, Itasca and St. Louis; 2) Montana - Glacier, Toole and Liberty; 3) Nevada – White Pine; 4) North Dakota - Burke, Divide, Mclean, Mountrail, Renville, Ward and Williams; 5) Oregon - Baker and Grant; 6) South Dakota - Custer, Fall River, Pennington and Shannon; and 7) Texas - Briscoe, Cottle, Dickens, Floyd, Hall, King and Motley.”</p>                             |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |
| <p>Page 6</p> | <p>“Each data set was then evaluated for significant differences between counties with and without <del>(89 sample sites located within 27 counties)</del> confirmed anthrax outbreaks or cases. This statement [yellow highlighted] is better explained in the results section; I don’t think you need it here.</p>                                                                              | <p>Deleted and moved to the end of the paragraph that now reads..... Anthrax-negative counties utilized for statistical evaluation included: 1) Minnesota – Aitkin, Itasca and St. Louis; 2) Montana - Glacier, Toole and Liberty; 3) Nevada – White Pine; 4) North Dakota - Burke, Divide, Mclean, Mountrail, Renville, Ward and Williams; 5) Oregon - Baker and Grant; 6) South Dakota - Custer, Fall River, Pennington and Shannon; and 7) Texas - Briscoe, Cottle, Dickens, Floyd, Hall, King and Motley. In summary there were 89 sample sites located within these 27 counties.</p> |
| <p>Page 6</p> | <p>“The non-parametric <b>Mann-Whitney U test</b> was utilized to evaluate differences in geochemistry between counties where <b>anthrax</b> outbreaks or cases had been reported since the year 2000 and counties within the same states where no cases were noted for the same time period using SPSS (IBM, Tampa, FL) (Dytham 1999).”</p> <p>Need a citation [for the Mann-Whitney U test]</p> | <p>Accepted and the Dytham citation is the proper citation for the test.....the Dytham stat book is one of the best out with full explanation of test selection, test purpose, how to enter data using various programs and how to interpret output data.</p>                                                                                                                                                                                                                                                                                                                             |
| <p>Page 6</p> | <p>“For statistical analyses those values were set at the minimum detection concentration for the</p>                                                                                                                                                                                                                                                                                             | <p>This was addressed in the Mano general comment above.</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |

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|                   | <p>respective elements (e.g. &lt;1 is set at 1)."</p> <p>How the elements that are present below detection limits are numerically handled may or may not have a profound effect on the analysis. Did you see if the statistical analysis results changed by setting the concentration of elements that were there at &lt; DL to Zero instead of DL?</p>                                                                                               |                                                                                                                                                                                                                                                                                                                                                                                                                             |
| Page 7            | <p>"These elements included calcium (<math>P &lt; 0.00</math>), niobium (<math>P &lt; 0.04</math>), nickel (<math>P = 0.03</math>), phosphorus (<math>P &lt; 0.03</math>), sulfur (<math>P &lt; 0.00</math>), tin (<math>P = 0.03</math>) and strontium (<math>P &lt; 0.05</math>)."</p> <p>Need more sig figs – P shouldn't be = 0.</p>                                                                                                              | <p>Unrounded these values and expressed them to down to 3 decimal points.....now reads.....These elements included Ca (<math>P = 0.006</math>), Nb (<math>P = 0.035</math>), Ni (<math>P = 0.028</math>), P (<math>P = 0.028</math>), S (<math>P = 0.002</math>), Sn (<math>P = 0.024</math>) and Sr (<math>P = 0.041</math>).....but there is &lt; 0.000 data in Table 1 as this is the output P value limit for SPSS.</p> |
| Page 7, line 144. | <p>"With the exception of niobium and sulfur, the overall average of elemental concentrations was higher in anthrax positive counties. When the elements were looked at individually, several trends emerged: "</p> <p>I found the following section difficult to follow in part because of the switching from element to element. I broke it down into a list to make it a little easier to follow-see</p>                                           | <p>Accepted.....good suggestion of breaking it down this way</p>                                                                                                                                                                                                                                                                                                                                                            |
| Page 7            | <p>"[New paragraph] <b>Strontium:</b> When contrasting the elements by each state, only strontium had average concentrations that were higher in all anthrax-positive counties versus anthrax-negative counties and the lowest observed concentration was 116 mg/kg."</p> <p>As written, this sentence contradicts what is written above (1<sup>st</sup> sentence says several elements were higher in anthrax positive counties, the second says</p> | <p>Accepted.</p>                                                                                                                                                                                                                                                                                                                                                                                                            |

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|        | <i>only strontium was higher). I think adding an "all" fixes it but please review</i>                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                                                                             |
| Page 7 | <i>"[New paragraph] <b>Calcium:</b> These concentrations were similar in both types of counties, with only one instance where average concentrations in negative counties exceeded positive counties and that was in Nevada at 5.05 and 3.03 wt. %, respectively."</i>                                                                               | Accepted.                                                                                                                                                                                                                                                                   |
| Page 7 | <i>"Overall, calcium data were significantly different in 3 of the 7 states."<br/><br/>Significantly different than what?</i>                                                                                                                                                                                                                        | Accepted.                                                                                                                                                                                                                                                                   |
| Page 7 | <i>"Phosphorus concentrations were similar to the average concentrations in Nevada (886 mg/kg) and Montana (827 mg/kg) were greatest in negative counties but these concentrations were the third and fourth highest overall concentrations in comparison to the data obtained from the other states."<br/><br/>This sentence is very confusing.</i> | <i>Changed to.....Phosphorus concentration averages in NV (886 mg/kg) and MT (827 mg/kg) were greatest in negative counties but these concentrations were the third and fourth highest overall concentrations in comparison to the data obtained from the other states.</i> |
| Page 7 | <i>"[New paragraph] <b>Phosphorus:</b> Phosphorus concentrations were similar as the average concentrations in Nevada (886 mg/kg) and Montana (827 mg/kg) were greatest in negative...."</i>                                                                                                                                                         | Accepted.                                                                                                                                                                                                                                                                   |
| Page 7 | <i>"Overall, phosphorus data were significantly different in 3 of the 7 states."</i>                                                                                                                                                                                                                                                                 | Accepted.                                                                                                                                                                                                                                                                   |
| Page 8 | <i>"[New paragraph] <b>Nickel:</b> Average nickel concentrations by state, with the exception of Montana, were higher in anthrax-positive counties...."</i>                                                                                                                                                                                          | Accepted.                                                                                                                                                                                                                                                                   |
| Page 8 | <i>"[New paragraph] <b>Niobium:</b> Significant differences in total niobium concentrations occurred with only...."</i>                                                                                                                                                                                                                              | Accepted.                                                                                                                                                                                                                                                                   |
| Page 8 | <i>"[New paragraph] <b>Manganese:</b> Manganese concentrations while not significant for the total data</i>                                                                                                                                                                                                                                          | Accepted.                                                                                                                                                                                                                                                                   |

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|         | set ( $P = 0.07$ ), were significant when contrasting counties....”                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                                                                                                                                                                                                           |
| Page 8  | “Manganese concentrations, while not significant for the total data set ( $P = 0.07$ ), were significant when contrasting counties in Texas, North Dakota, South Dakota, Minnesota and Nevada.”                                                                                                                                                                                                                           | Accepted.                                                                                                                                                                                                                                                                                                                                                 |
| Page 8  | “[New paragraph] <b>Sulfur</b> : The total sulfur significant difference....”                                                                                                                                                                                                                                                                                                                                             | Accepted.                                                                                                                                                                                                                                                                                                                                                 |
| Page 8  | “The total sulfur significant difference (high concentrations in negative counties) occurred over a small concentration range (0.02 to 0.19 wt%) and the only state-level significant difference that occurred was with the Texas data set and that was opposite (high concentrations in positive counties) the total.”                                                                                                   | Accepted.                                                                                                                                                                                                                                                                                                                                                 |
| Page 9  | “This observation was also noted with the <b>sulfur S</b> data.”                                                                                                                                                                                                                                                                                                                                                          | Accepted.                                                                                                                                                                                                                                                                                                                                                 |
| Page 9  | “For <b>manganese Mn</b> , there was one negative (due to the second highest average concentration at 1144 mg/kg, relative to the overall seven-state data set average of 808 mg/kg) and four significant positive state data.”                                                                                                                                                                                           | Accepted.                                                                                                                                                                                                                                                                                                                                                 |
| Page 10 | <p>“Also of note are elements such as barium and rubidium, which produced multi-state negative significance data, may inhibit virulence by mechanisms such as mimicking a critical virulence element (e.g., zinc).”</p> <p>Is there a citation for this? It's really hard to definitively identify these types of effects from field data, but there may have been a fundamental study somewhere that did look at it.</p> | Added the following citation of how barium mimic or co-compete with calcium in cells as an example....Heldman E, Levine M, Raveh L, and Pollard HB. (1989) Barium ions enter chromaffin cells via voltage-dependent calcium channels and induce secretion by a mechanism independent of calcium. <i>Journal of Biological Chemistry</i> , 264, 7914-7920. |
| Page 10 | “It is interesting (as can be observed in supplemental-Figure 1) that the concentrations of both of these elements are relatively low in many of the anthrax- <b>positive</b> <del>not</del> counties of N. Dakota,                                                                                                                                                                                                       | Accepted.                                                                                                                                                                                                                                                                                                                                                 |

|         |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                                                                                                                                                                                                                                                      |
|---------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|         | <i>S. Dakota, Minnesota and Texas."</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                                                                                                                                      |
| Page 10 | <i>"<del>These concentrations can be utilized</del> We can utilize these as thresholds for an investigative tool to determine the likelihood of a naturally occurring outbreak being 'potential' at 0.43 wt. % or above and 'probable' at 1.3 wt. % or above."</i>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | <i>Along with reviewer comment changed to ..... These concentrations can be utilized as putative thresholds for an investigative tool to determine the likelihood of a naturally occurring outbreak being 'potential' at 0.43 wt. % or above and 'likely' at 1.3 wt. % or above.</i> |
| Page 11 | <p><i>"Using concentrations observed at sample sites in the states listed in Table 1 for Ca, Mn, P and Sr, several tentative threshold concentrations can be set for each element in regard to the likelihood of an outbreak occurring at a given location. As an example, the minimum concentration observed in any of these state counties for calcium is 0.43 wt. % and the lowest significant average listed in Table 1 is 1.3 wt. %. We can utilize these as putative thresholds for an investigative tool to determine the likelihood of a naturally occurring outbreak being 'potential' at 0.43 wt. % or above and 'probable' at 1.3 wt. % or above. Similarly, 'potential' and 'probable' thresholds can also be set for manganese (144 and 463 mg/kg), phosphorus (180 and 580 mg/kg), and strontium (51 and 170 mg/kg). Figure 3 illustrates those sample sites where those upper or 'probable' concentration levels occurred both individually and in combination."</i></p> <p><i>Comment referring to yellow highlighted text: More likely? What do you mean by probable? Are the odds better than 50% that there will be an outbreak at a given location.</i></p> <p><i>Comment referring to this paragraph: Since this is an</i></p> | <i>Adopted and changed probable to likely throughout the document.</i>                                                                                                                                                                                                               |

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|                      | <p>observational study, this paragraph seems to be a big leap. Threshold concentrations can be identified for further evaluation, but it seems premature to use this data to calculate the likelihood of an event; wouldn't you need to look at some other counties first and confirm your data?</p>                                                                         |                                                                                                                                                                                                                                                          |
| Page 11              | <p>"Evaluation of geochemical data from <del>the</del> a series of selected sample sites in XXX states identified four elements that had significant positive differences in concentrations of the total data set or in a majority of the states utilized for analyses. "</p> <p>Confusing phrase – please clarify</p>                                                       | <p>Changed to read....<br/>Evaluation of geochemical data from a series of selected sample sites in seven states identified four elements that had significant differences in concentrations between anthrax-positive and anthrax-negative counties.</p> |
| Page 11              | <p>"Threshold values based on the lowest concentrations and the lowest average concentrations of each of these elements, in the anthrax positive counties utilized in this study were identified for use in prospective tools for determining whether or not a naturally occurring outbreak is 'potential' or 'probable' at any given geographic location."</p>              | <p>Changed to 'use as.'</p>                                                                                                                                                                                                                              |
| Page 11, conclusions | <p>"Threshold values based on the lowest concentrations and the lowest average concentrations of each of these elements, in the anthrax positive counties utilized in this study were identified for prospective tools for determining whether or not a naturally occurring outbreak is 'potential' or 'probable' at any given geographic location."</p> <p>More likely?</p> | <p>Changed to 'likely.'</p>                                                                                                                                                                                                                              |
| Page 12              | <p>"We <del>The</del> authors would like to thank Sarah Perkins formerly of the USEPA for her help and assistance on this project."</p>                                                                                                                                                                                                                                      | <p>This is the reverse of what the other reviewer suggested....Changed it from 'we' to 'the authors' as that reviewer suggested</p>                                                                                                                      |
| Table 1              | <p>This table is really hard to understand. Maybe use some color coding to help clarify which elements were statistically</p>                                                                                                                                                                                                                                                | <p>See next response.</p>                                                                                                                                                                                                                                |

|                            |                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                            | <i>significant? Maybe white, yellow, and green.</i>                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <i>Table 1</i>             | <i>I had several questions about the table-why are the P values only listed in some cells and not others? (eg Ca/Oregon). Why are the average concentrations listed in some cells and not others (eg La/Nevada). I assume .000 means no element was detected in any samples? I also assume the empty cells mean not done? I think the cells in the table should be more consistent with regard to the data listed.</i> | <i>Changed to title of the table to explain that...to read... Table 1. The significance (Mann-Whitney U test P-values – in bold where &lt; 0.05) of elemental concentrations (averages in brackets [#/#] where an overall or greater than two state significant P-values) in counties reporting outbreaks or cases of anthrax in livestock and wildlife versus counties that have not reported outbreaks or cases of anthrax since the year 2000.</i> |
| <i>Figure 2</i>            | <i>Text is too small to read.</i>                                                                                                                                                                                                                                                                                                                                                                                      | <i>Enlarged.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <i>Supplemental figure</i> | <i>Supplemental Figure 1. Barium and <del>Robidium</del> Rubidium soil concentration heat maps for the contiguous United States.</i>                                                                                                                                                                                                                                                                                   | <i>Accepted.</i>                                                                                                                                                                                                                                                                                                                                                                                                                                      |
| <i>References</i>          | <i>References should be before the figures and tables and supplemental information.</i>                                                                                                                                                                                                                                                                                                                                | <i>Yes, upon submission.</i>                                                                                                                                                                                                                                                                                                                                                                                                                          |

## The Dissemination

The published information product will be released in the open literature as a journal article. The primary target journal will be *Science*.