RESEARCH PLAN

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TITLE: Elemental Composition of Fossilized Ceratopsidae and Dromaeosauridae Teeth from the Lance Formation, Wyoming, USA

RATIONALE:

- **Background**
    - The purpose of paleontology, as a science, is to advance the understanding of the ecological, geological and evolutionary patterns that took place millions of years ago. Paleontological research has evolved past the understanding of dinosaur anatomy, and has grown into a multidisciplinary science that aims to visualize and understand what life on earth was like prior to the human race (Zeigler 2014). Specifically, both biogeochemistry and chemical paleontology have seen significant development since the beginning of the 20th century (Bocherens 1997). Despite the advancement of these sciences, there are still countless gaps concerning all aspects of the interactions and developments of prehistoric life. Understanding the dietary patterns of dinosaurs has always been a focal point in the advancement of paleontology, which is a possibility of learning through studying teeth. In order to investigate these dietary patterns, scientists reference either coprolites — which are fossilized animal stool — or fossilized organs such as gastric mills or stomachs. Other methods for identification of eating patterns are the analysis of tooth morphology/morphometry, tooth microwear, or the physical anatomy of the dinosaur (Zanno and Makovicky 2010, Samman et al. 2005, Williams et al. 2009, Mallon et al. 2013, Zhou and Zhang 2002, Zhou et al. 2004). However, there are more extensive ways that can possibly identify dietary patterns for single animals, rather than a general census across a whole species which most of those methods listed above are only capable of doing (Zanno and Makovicky 2010). Fossilized dinosaur teeth are some of the only existing specimen from dinosaurs that are composed of the same elements now, as they were millions of years ago. Teeth act as the best retainers of both ecological and biological information, which ultimately makes them the most optimal specimen to use to analyze dietary patterns, climate, and ecology (Bocherens 1997). With the teeth being some of the best preservers of information and constituents from over 66 million years ago, when analyzed, they have the greatest capability to paint a picture of what the climate, ecology, and setting of the Earth was like as these animals roamed the Earth.

- **Scientific Importance / Societal Impact**
    - In the Lance Formation of Wyoming, there has been a limited amount of research performed due to issues with land access and ownership. In relation to the extensive examination of the Hell Creek Formation, another Cretaceous era site that stretches over a majority of the Badlands, there is a dearth of research in the Lance Formation. Thus, there is already an innate, immense gap in this research in the Lance Formation as compared to surrounding areas. To expound upon this gap, only a limited number of biogeochemistry or chemical paleontology studies have been performed on the Lance Formation. The introduction of an elemental analysis of teeth at the Lance Formation would add to the cache of knowledge on dinosaur life just prior to the Cretaceous-Paleogene extinction event.
    - In paleontology, there are plentiful sects of the science that have rarely been addressed. The development and availability of advanced resources have caused for there to be an increase in research possibilities. Specifically since the conception of such advanced methods to analyze the elemental composition of specimen, the resource has been utilized for varying projects under the Synchrotron capabilities, although few of them analyze teeth.
    - This method will not only be unique to research based from the Lance Formation, but also introduces an insight to the capabilities of synchrotron technologies, which have not fully been utilized by the paleontological community.

RESEARCH QUESTION / HYPOTHESIS:

Research Question

- Can ecological attributes of Pre-Creteaceous species be deduced by elemental analysis from the teeth of herbivorous non-avian dinosaurs (*Ceratropsidae*) and carnivorous non-avian dinosaurs (*Dromaeosauridae)*?

Hypothesis/Expected Outcomes

- We expect to see a difference in the elemental composition of the *Ceratopsidae* tooth and the *Dromaeosauridae* tooth, based on various factors that could affect the composition of teeth whilst living, and compare those elements to geological samples isolated from the same location.

PROCEDURES

Sample Collection:

Samples will be collected at the Zerbst Ranch Microsite, Lance Formation, Wyoming in July 2019. Samples will be consistently handled with gloves and clean plastic tweezers to avoid contact with metals. To extract the sample, the researchers will use sieves, tweezers, or gloved

hands. Basic identification will be performed in the field to guarantee that the samples collected suffice the goals of the project, in which we needed a tooth of *Ceratopsidae* descent and one *Dromaeosauridae* tooth. During travel, tooth fossils will be wrapped in KimTech cloths and placed into 100 mL vials individually, and carried with precaution in carry-on bags. Fossil identification will be confirmed by use of field guides.

Sample Preparation:

For preparation, both teeth will be lightly brushed to remove all rock and sediment from the surface of the sample. Samples will be mounted together on specialized SRX holders provided by the Brookhaven National Laboratory (BNL) and will be reinforced using Kapton® Tape to support the samples. Samples will be arranged so that their flat side is facing the beam, outwards from the holder, as it is a necessity that the topography is flattest facing the beam so the focus can be constant.

SRX-Specific Methods:

Samples will be placed in the hutch of SRX and held using magnets. SRX will be used at the incident energy of 16.5 keV, which will allow the researchers to see the elements with incident energies from 4.65 to 16.5. The beamline will then be calibrated to optimize the results. Samples will have a scanning area of 200 microns squared (μm^2). The beam size will be $1 \mu m^2$ and the pixel size will be 2 μm^2. Scans will be administered for each tooth after the optimal scanning location is chosen. Once one sample completed its' run, the shutter will be closed and the second scan will be setup and run.

Risk and Safety:

- While the site of collection of the fossils is near the road and mostly flat, the researchers will wear proper sturdy footwear, gloves and safety goggles at all times when excavating. A certified medical professional will be with our group at all times, and our guides carry a first aid kit at all times. We will also carry water at all times and minimize exposure time to the sun. At all times we will follow our supervisors guidelines, local and federal laws and minimize our impact to the surrounding environment.

- The NSLS-II has various potential hazards, such as exposure to radiation. All minors present on floor of the NSLS-II are required to wear thermoluminescent dosimeter (TLD)'s, which are devices that measure intake dosage of any radiation. These values were computed by BNL, and regulated by the United States Department of Energy. In addition, BNL requires that any researcher that applies for access to a resource fully completes multiple on-line safety courses prior to any on-site training to ensure the safety of the users and information at BNL. Upon arrival to SRX, the researchers will be given a beamline-specific training that is facilitated and instructed by the beamline scientist. At

all times, either the lead experimenter, head beamline scientist, or other beamline scientists are present to facilitate all activities and ensure the safety of the researchers and facility. (All actions are closely monitored) The beamline scientist works with/guides the researchers during the operation of the beamline in order to guarantee safety. To further the decrease of radiation risk, the hutch is enclosed and contained in a manner that makes it virtually impossible to come into contact with radioactivity.

DATA ANALYSIS

pyXRF:

- After the scans are completed, the data will be analyzed using pyXRF. Each sample will be made into a Spectrum Map, individual Elemental Maps, and overlaid Red-Green-Blue (RGB) Maps, which are three elements superimposed upon each other in order to see homogeneity, heterogeneity, or other relationships between the elements in the sample. Figures are then to be compared to look for differences between the two teeth to view if there are elemental differences and compared to the rock sample. The Elemental Maps and RGB Maps will be examined closely to see patterns in the teeth individually because there are colocalizations of certain elements and localities of extreme intensities, among other patterns to list as well. The Spectrum Maps will surely offer a visual of the counts of each element present, which makes it easier to understand the elements that are present and their intensities. Spectrum Maps will then be converted into Google Sheets to create more presentable and understandable figures.

Addendum: Four additional teeth – two from each species – were added to the study to increase the data set and create a better representation to each species.

4.Hazardous chemicals, activities & devices:

- There was no additional risk involved.

Addendum: Depositional rock from the Zerbst Ranch Microsite was analyzed in order to act as a negative control.

4.Hazardous chemicals, activities & devices:

- There was no additional risk involved.

Addendum: While our initial plan relied solely on the SRX beamline, the opportunity for analysis on three other beamlines of the NSLS-II at BNL were offered to the researchers. Data from TES and XFM were all collected, analyzed, compared, and added to the cache of data that we had already collected from SRX.

4.Hazardous chemicals, activities & devices:

- TLD's which measure monthly exposure to radiation were required to be worn whilst at the NSLS-II. Researchers went through extensive training both online in security and safety, as well as beamline-specific training in order to guarantee that all researchers are aware of what to do in case of an emergency. Head beamline scientists trained the researchers and stayed on the experimental floor in order to oversee experimentation for the duration of the experiment.

Addendum: Two teeth were polished using a diamond blade at the TES Preparation Wet-Laboratory at the NSLS-II at BNL. This granted the researchers the opportunity to view past the enamel, into the dentine layer of the tooth.

4. Hazardous chemicals, activities & devices:

- As this would require surplus training and add another increase in risk, the samples were left with Brookhaven National Laboratory scientists in order for this step to be done. The samples were cross-sectioned by professionals that are certified to use this equipment, so there was no additional risk factors added for the researchers.

Addendum: Additional software was used including Interactive Data Language (IDL) data-analysis software at TES, GSE MapViewer at XFM and a program drafted by the researched to further investigate the mean pixel value for selected elements of each sample.

4. Hazardous chemicals, activities & devices:
   - There was no additional risk involved as this is a common practice performed on a laptop.

Addendum: Certain samples were re-mounted multiple times throughout the process to best optimize the capabilities of the beamlines of the NSLS-II.

4. Hazardous chemicals, activities & devices:
   - The remounting of samples leaves little risk to safety, but the researchers did conduct themselves properly in the laboratories so that they did not damage any equipment or cause bodily harm. All work was done with the same safety precautions that have been followed all along.

Addendum: The researchers sought further identification of the teeth through both fossil guides and personal communications with paleontologists.

4. Hazardous chemicals, activities & devices:
   - Personal communications have virtually no possible risk-factors.