

Determination of Uranium in Sheep (*Ovis aries*) Kidneys by Inductively Coupled Plasma Mass Spectrometry (ICPMS)

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Abstract

Abandoned Uranium Mines (AUM) are the main source of anthropogenic uranium contamination on the Navajo Nation [1]. In parts of the Navajo Nation, AUMs have negatively impacted the environment and people since the 1950's [2]. The main routes of exposure are the ingestion of contaminated drinking water and inhalation of dust blow particulates, with the kidney being recognized as a site for uranium (U) accumulation in the body along with the skeleton and the liver [3]. Previous research reported characterization of U concentrations in sheep, *Ovis aries*, grazing near AUMs, a decommissioned U milling site, and downstream of a major U tailings pond spill that occurred in 1979 [4,5]. Results from previous studies suggest low accumulation in tissues and organs. However, little is known about the accumulation of U in sheep kidneys based on their exposure to different types of AUMs (e.g., open pit, underground). The purpose of this study was to characterize the concentrations in the kidneys of sheep grazing on or near AUMs on the Navajo Nation. Sheep sampled for this publication grazed near the community of Cameron, AZ (N=4), a community that experienced open pit mining in the 1950's, and the community of Cove, AZ (N=4) a community that experienced underground mining in the 1950's. Sheep selected for inclusion this study were euthanized and their tissues collected for analysis. A reference site was used as there is not a Standard Reference Material (SRM) for U in sheep, therefore the reference site was a community roughly 100 miles southeast of the Navajo Nation in Eagar, AZ. ICPMS analysis was used to determine kidney U concentrations. Statistical analysis comparing kidney U concentrations between mining and non-mining areas was performed using Kruskal Wallis and Dunns' Test. Results suggest U concentration differences were found between the mining sites versus the reference site. Additionally, there was no statistical difference between the AUM types. Based on accounts from ranchers, sheep from Cameron were grazing near/on open pit mine sites, while half of the sheep from Cove grazed in a canyon that had mine waste runoff from underground mining present.

Keywords: *Ovis aries*; Sheep; Heart; Uranium; Navajo

Abbreviations: AUM: Abandoned Uranium Mines; U: Uranium; AEC: Atomic Energy Commission; NAU: Northern Arizona University; IACUC: Institutional Animal Care and Use Committee; PPB: Parts Per Billion; VWR: Voluntary Work Reduction; ICP-MS: Inductively Coupled Plasma Mass Spectrometer; SD: Standard Deviation; MCL: Maximum Contaminate Level

Introduction

The average content of the earth's crust is approximately four grams U per ton (g/t) of crustal material. [6,7]. Uranium (U) mining started in the United States in 1948 following the US Atomic Energy Commission (AEC) guaranteeing a price and purchase for all U in the United States [8]. From 1948 to 1982, the US government was the sole purchaser of U [9]. By 1958, there were 7,500 reports of U claims in the US with roughly 7,000,000 tons of ore identified and 750 mines in operation [10,11]. This announcement initiated a rush for U mining, especially in the American southwestern, specifically Arizona, Colorado, and

Utah [12,13]. U outcrops were first reported in Cameron in the 1950's, with mining peaking in 1957. By the end in 1963, a total of 289,300 tons were removed from 98 open pit properties [14]. Open pit mining is the process of extracting rock or minerals from the earth through their removal from an open pit or borrow [15]. U was first reported in Cove in 1949 and mining began in 1950, reaching peak production in the 1960's. A total of seven million tons was removed from 50 separate underground U mine sites by the time mining ended in Cove in 1968 [14]. However, anthropogenic activities like mining can result in opportunities

for humans, plants, and animals to be exposed to U [16]. U mining activities in these communities commenced at approximately the same time but above ground pits were prominent in the Cameron community while underground mining was prevalent in the Cove community.

Abandoned, unremediated U mines sites remain in more than 50% of the governing units (e.g., chapters) on the Navajo Nation [17]. High levels of U are still found in soils, plants, and water sources around these U mines. Studies suggest waste products from U mining provide a mechanism for U uptake in plants materials, thus posing a potential harm to animals like livestock as abandoned U mine sites on the Navajo Nation are commonly unfenced, unmarked, and still used for livestock grazing [17-19]. Navajos rely on sheep as well, a traditional food staple, all aspects of the sheep are used, and there are important cultural uses for sheep [20]. Studies conducted in the Navajo communities of Cameron and Cove focused on understanding consumption of mutton, led by Rock and Nez confirmed that if Navajos could eat more sheep they would take that opportunity, and if sheep are absent that it disrupts family gatherings and ceremonies [21,22]. Mutton consumption studies bring to light that sheep are an important food to the Navajo and understanding the accumulation of U in sheep is crucial [21,22]. This may suggest a potential exposure for human consumers, since sheep meat, organs, and other edible parts of the sheep form the basis of many popular traditional Navajo meals, especially in rural areas of the Navajo Nation. Exposure to the metals and metalloids found in U mine waste, either directly from the waste or through meat and organ tissue from livestock, may lead to health risks for both domestic animals and humans [23]. U enters the body through numerous pathways including the inhalation of windblown U laden dust, the oral intake of U contaminated water, food, and soil in U polluted areas [24]. The accumulation of U in livestock tissue may also pose a risk to human health from meat and target organs destined for consumption [16,25,26]. Following absorption, U is widely distributed in the body and excreted primarily in the feces and urine. The common distribution of U is within the skeleton, liver, and kidney [25]. Toxins are generally cleared from the body by either the kidney, liver, or gastrointestinal tract. The kidney is required and responsible for maintaining total body salt, water, potassium, and acid-base balance, while eliminating waste products and toxins, and preserving the body's internal environment [3]. The kidney is recognized as a major site for U accumulation [17]. The potential functional and structural targets of U toxicity include cell types involved in filtration (glomeruli, tubule system) and concentration of urine (collecting tubule and ducts) [27]. U induced kidney toxicity in mammals is caused by the precipitation of hexavalent U mostly in the kidney tubules during the urinary blood clearing process [28]. *Consequently*, tissue damage leads to kidney failure with all typical pathologic findings in the blood and urinary system.

Other studies quantified U accumulation in the heart and target organs of domestic sheep, Lister reported U concentrations in the heart of sheep grazing near abandoned U mine sites in Cameron AZ to be consistent with other studies in the literature [29]. The similarities of U concentration in sheep hearts collected in Cameron and the reference site illustrates the complexities of U uptake in sheep on the Navajo Nation (e.g., tracking the origin of animals and tracking the contamination of U on the southwestern part of the Navajo Nation) [23,30]. This study, coupled with a similar study conducted by Nakamura, suggests that sheep from mining areas on the Navajo Nation chronically exposed to U are accumulating U in the target organs (e.g., kidney). In past studies, U has been found to accumulate in sheep grazing on or near abandoned U mine sites; however, the impact of the type of U mining (e.g., open pit, underground mining) on livestock is unknown. We hypothesize that sheep grazing near the open pit mining areas in Cameron will have higher concentrations in the kidney that those grazing near the underground mining areas in Cove due to the larger surface areas of exposure from the abandoned open pit mines. The purpose of this study was to 1) determine the concentrations of U in sheep kidneys that grazed in two chapters on the Navajo Nation that included AUMs; and 2) compare measured concentrations between U mining type and to the control area.

Methods

Sample collection

For this publication, we will focus on the chemical aspect of U accumulation in sheep kidney. Approval for all procedures employed in this study were granted by the Northern Arizona University (NAU) Institutional Animal Care and Use Committee (IACUC), NAU Institutional Review Board, Navajo Nation Human Research Review Board, and by the communities of Cameron, Cove. Samples were collected from Cameron in the summer of 2013, and samples were collected from Cove in the summer and fall of 2019. Approval was also obtained from a rancher from Eagar, AZ (the reference site for this study). Samples were collected in the fall of 2015 and spring of 2016 from Eagar. The addition of a reference site was needed as there is not a standard reference material for U in livestock. The community of Eagar is located 100 miles from the Navajo Nation and does not have a history of U mining (Figure 1). The communities of Cameron and Cove are located on the Navajo Nation, Cameron is 53 miles northeast of Flagstaff, AZ, and Cove is 237 miles northeast of Flagstaff (Figure 1). The age range of the Cameron sheep (N=4) was two to six years; the Cove sheep (N=4) were one to two years; and the age range of the Eagar sheep was one to eight years. The aging of the sheep was conducted by veterinarians Dr. Adrienne Rudy and Dr. Holly Johnson-Grahams.

Sample preparation and analytical methods

All the sheep were euthanized using a traditional Navajo butchering technique at the stie where the animals were raised

[31]. Kidneys from the sheep euthanized were collected for this study, upon collection kidneys were placed in gallon sized freezer bags, placed in an ice chest, and transferred back to NAU to be

prepared for sample analysis. At each site a chain of custody sheets was created to ensure both kidneys were collected from sheep that were euthanized.

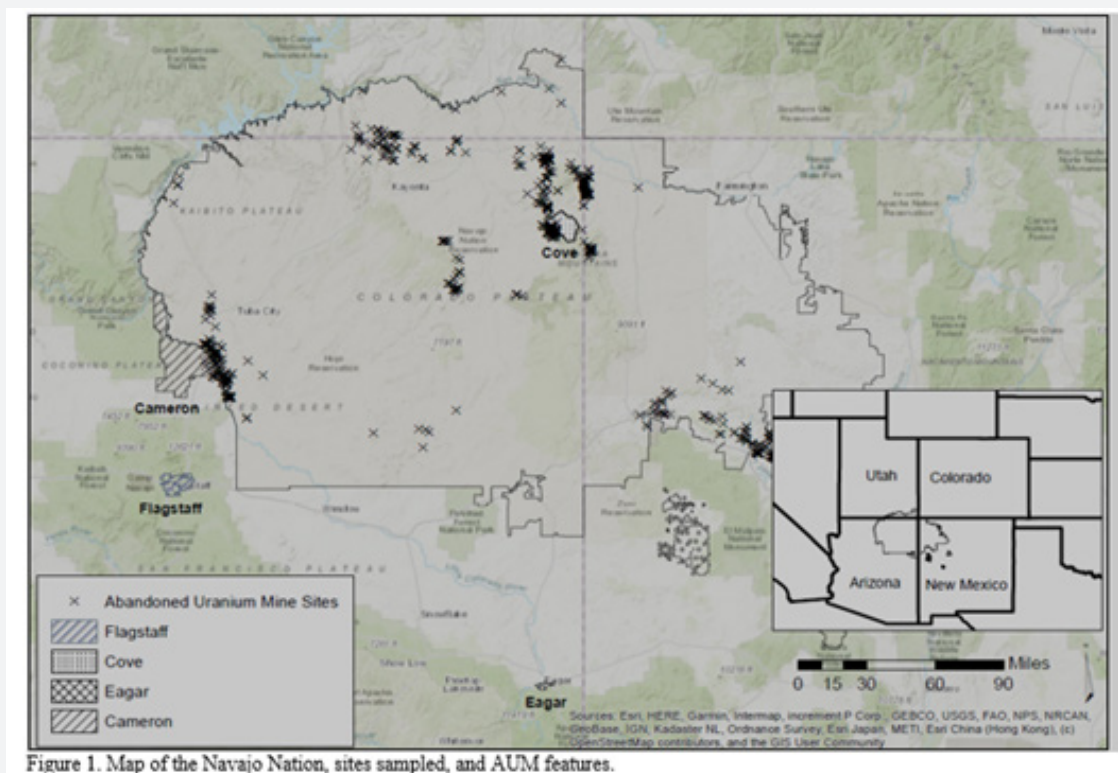


Figure 1: Map of the Navajo Nation, sites sampled, and AUM features.

Sample preparation includes slicing kidney tissue samples as thin as possible, allowing them to dry for two to three weeks, powdering in standard coffee kitchen coffee grinder, and homogenizing in a sterile 500 mL Whirl Pak sample bag (VWR). The coffee grinder was taken apart, soaked in high purity deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$, Thermo Genpure Pro), and each individual part was cleaned with dish soap (Dawn), rinsed with high purity deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) three times, and set out to dry. The samples were separated into three replicates from each sample. Exactly, 13.0 g of sample were placed in a 10 mL ceramic crucible (VWR) and mineralized for 24 hours using a muffle furnace (Thermo Blue) programmed with a temperature ramping method to remove the organic matter. Temperature ramping allows the sample to turn to ash rather than burn up and melt into the crucible. Mineralized kidney tissue sample was transferred to 50 mL centrifuge tubes for partial digestion in 25% omni-pure grade nitric acid (HNO_3 , VWR) and brought to volume with high purity deionized water ($18.2 \text{ M}\Omega\cdot\text{cm}^{-1}$) to 50 mL. The samples were diluted 1:5 by mixing 4 mL with dilution solution

containing 0.05 ng/L ruthenium internal standard (El Chrome), and 1% HNO_3 and high purity deionized water.

A Thermo X-series II inductively coupled plasma mass spectrometer (ICP-MS) with conical spray chamber (Analytical West), equipped with a c-type nebulizer 1 mL/mi (Analytical West), was used for U quantitation. Argon gas flow rate was set to 11.9, auxiliary flow of 0.70, and nebulizer flow at 0.83 L/min. A calibration curve was obtained with a minimum correlation coefficient (R^2) value of 0.997 prior to continuing analysis. U calibration standard concentrations were 0.0, 0.1, 0.25, 0.50, 1.0 parts per billion (ppb), and an internal standard of 0.05 ppb ruthenium was used. The sample concentrations and extraction efficiencies were determined from the $\text{U}238$ to $\text{Ru}101$ mass to charge ratio.

Statistical analysis

Summary statistics for sheep kidney U concentrations were calculated for each sample group including minimum, maximum

range, mean, median, and standard deviation (SD). Non-parametric test statistics were calculated due to the small number of samples per group and the non-normal distribution of the results. Specifically, the median sheep kidney U concentrations among open pit, underground, and no mining were elevated for significant difference using the Kruskal-Wallis test. If the test result was significant (p-value, 0.05) then a Dunn's test was conducted to identify pairwise differences between groups. Data was evaluated using R studio (version 2002.07.01) and the following packages (FSA: Ogle DH, Doll JC, Wheeler P, Dinno A (2022). FSA: Fisheries Stock Analysis. R package version 0.9., dplyr version 1.0.7, ggplot2 version 3.3.5, multicopy View version 0.1-8) were used in the statistical analysis of the data.

Results

The present study investigated concentration of U in the kidney of sheep that grazed in Navajo communities near abandoned U open pit and underground mining sites and reference area with no history of U mining. Analytical results indicated that the concentration of U in sheep kidneys among the three groups ranged from the lowest at 34.27 (SD = 9.13) and the highest at 176 (SD = 524.86) ng/g ashed mineralized weight (Table 1). The kidneys of the sheep from the open pit U mining contained mean value of 149.27 (SD = 52.42) ng/g ashed-mineralized weight, and the kidneys of the sheep from underground U mining contained mean value of 176.35 (SD = 242.86) ng/g ashed-mineralized

weight. The underground mining location had three sheep with concentrations with a mean value of 42.18 (SD = 6.14) ng/g ashed mineralized weight; however, there was an outlier, sheep four kidney from Cove that had a mean concentration of 578.84 (SD = 15.54) ashed-mineralized weight ng/g. Boxplots of the results visualize the median and interquartile range for each group of animals (Figure 2). Overall, the group U kidney concentrations were higher for the Cameron sheep compared to the other groups; however, the highest measurements were reported for a sheep from the Cove community. For additional comparison, the two mining subgroups were aggregated into a single group that was compared to the non-mining location (Figure 3). The two mining subgroups had a mean concentration of 162.81 (SD = 172.37) ashed mineralized weight ng/g and the non-mining location had a mean concentration of 34.27 (SD = 9.13) ashed-mineralized weight ng/g.

Table 1: U concentrations in sheep kidney tissues from three sites in Arizona.

	Mining	Mean	Median	Standard Deviation
Cameron	Open pit	149.28	148.14	52.42
Cove	Underground	176.35	46.39	242.86
Eagar	Reference	34.27	34.09	9.13

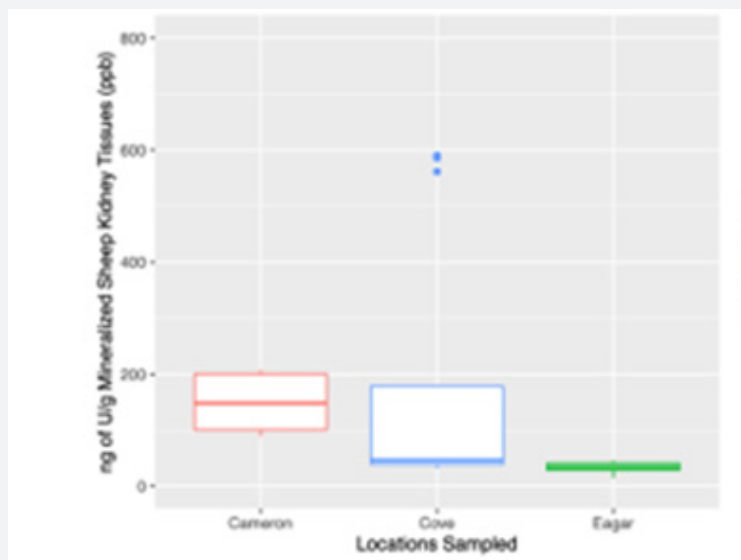


Figure 2: (Left) U accumulation in sheep kidneys from three different communities in Arizona.

Results from the Kruskal Wallis test suggested a difference in medians among the three sites (p-value = 4.37×10^{-5}) [32]. Dunn's test was used to test for stochastic dominance among the three groups following a Kruskal Wallis test, thus Dunn's

test suggested there was a statistical difference among all the sites and their mean values [32]. Additionally, results from the Dunn's Test suggested U accumulation in the sheep kidneys from U mining sites were not similar (e.g., open pit, underground), and

comparing the reference site to the both the mining sites there were no similarities as well. Results from a Kruskal Wallis test suggested there was a difference between the combined mining sites versus the reference site ($p\text{-value} = 4.24 \times 10^{-5}$). A Mann-

Whitney U test was used to determine the difference between the aggregated mining group and the reference group [33]. As a result, the Mann-Whitney U test suggested the aggregated mining group was not like the reference site ($p\text{-value} = 6.75 \times 10^{-6}$).

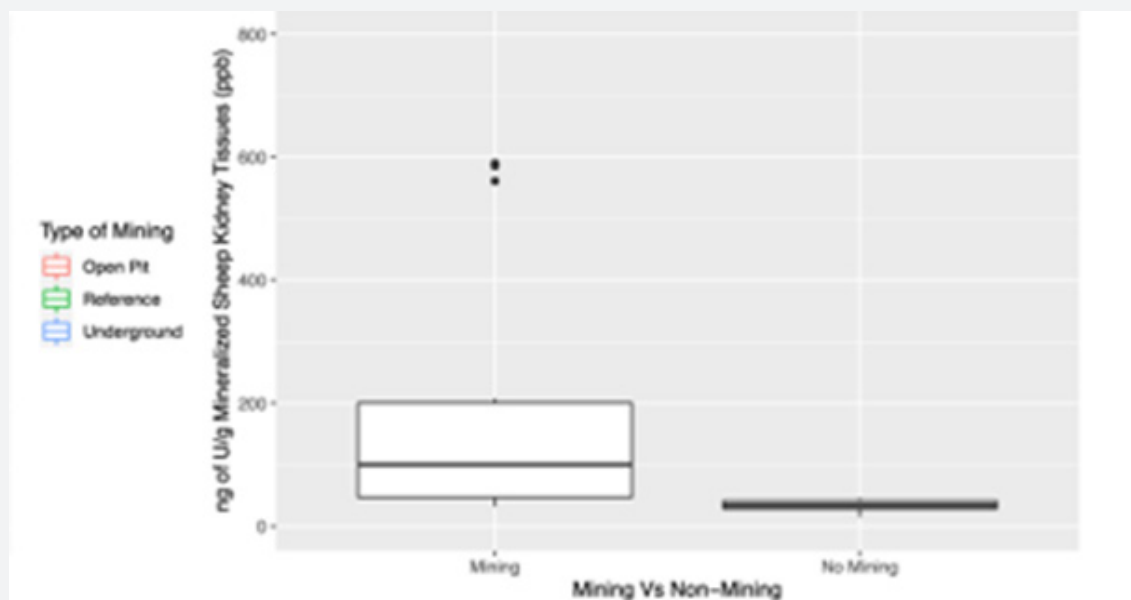


Figure 3: (Right) Comparison of U accumulation in sheep kidneys from mining areas to non-mining areas.

Discussion

Based on interviews with Cameron community members that provided the sheep for the study did not know the approximate grazing habits and histories of the Cameron sheep, thus the exact exposure of the sheep to AUMs in the community is unknown. Additionally, in talking with community members, Cameron ranchers traded sheep with ranchers in nearby communities, again, there are uncertainties with how long the sheep grazed in the communities of Cameron. The Cove sheep were fitted with geospatial units that tracked their approximate grazing habits for several months, thus the grazing habits for the sheep were known before the sheep were euthanized. One of the Cove owners had his sheep graze near his home and supplemented with hay and from nearby Navajo Agricultural Products Industry (NAPI), while the other owner grazed their sheep in the canyons that had mine waste run off present in high concentrations throughout the canyon. The Eagar community that provided the reference sheep for the study communicated that the sheep provided for the study lived on a hay-based diet, and were corral kept.

Veterinarians estimated the ages of sheep based on their teeth, this is a rough estimate as the condition of teeth will vary according to the type of feed and landscape grazed on. For

example, if sheep graze on long, soft feed (e.g., long grasses and little exposure to soil) the teeth will grow long from lack of wear and will remain in good condition [34]. However, if the sheep graze on short feed (e.g., short grasses, and more exposure to soil, gravel, and rocks), the teeth will wear down [34]. Cameron is in an arid region of the Navajo Nation with minimal vegetation, Cove is also located on the Navajo Nation surrounded by the Lukachukai and Carrizo Mountains. Eagar is located off the Navajo Nation, near Springerville and the White Mountains which is known for its rolling grasslands and forest [35]. *Consequently*, landscapes like Cameron are problematic in aging the teeth of the sheep because the lack of vegetation and increase exposure to soil, gravel, and rocks can wear the teeth of the sheep, potentially, leading to an over estimation of the age of the sheep.

The existing literature does not report U maximum contaminate level (MCL) for U in sheep kidneys. In this study, both kidneys were homogenized, dried, powdered, acidified, and analyzed with ICPMS. Results suggest the sheep that grazed in Cameron have higher concentrations due to the ingestion U contaminated pasture and soil from past open pit mining activities in comparison to the other sites in this study, which corroborates out hypothesis that sheep grazing near open pit

mining areas will have higher U concentrations in their tissues. Comparing the concentrations of this study to other studies, this study had higher U concentrations to that of the Nakamura et al. [4] sheep study. Additionally, comparing this study to other ruminant studies in different parts of the world, U concentrations in sheep kidneys analyzed for this study were much higher than the U concentrations found in caribou (14 to 18 ng/g dry weight) and cattle (8.8 ng/g dry weight) kidneys from Northern Canada and East Germany [23,36]. Overall, the results suggest a need to create health-based thresholds for consumption that protect both livestock and human health.

Conclusion

In this study, the accumulation of U in sheep kidneys was measured by ICPMS. Results suggest that sheep grazing in communities with U mine sites whether they be open pit or underground, have higher kidney tissue concentrations of U than sheep from areas with no history or U mining. Comparing the U kidney concentration in this study to other ruminants' kidneys (e.g., sheep, caribou, cattle) in different parts of the world (U.S, Canada, Germany), the concentrations in this study were higher [4,23,26]. This study is an ongoing study, future publications will further address some of the limitations of this initial publication. The human health risks of eating locally grown sheep being chronically exposed to U from past mining remains unknown. Results from this study support the community hypothesis that sheep grazing on or near abandoned U mines are exposed to U, thus leading to negative implications on the consumption, culture, and tradition of the Navajo [21,22]. Nonetheless, this ongoing research will continue addressing U accumulation in sheep work and is a step towards addressing long standing U mining legacy problem. Future work will lead to a refined understanding exposure, support interventions that will reduce livestock and human U exposure, and ultimately lead to healthier people and communities on the Navajo Nation.

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