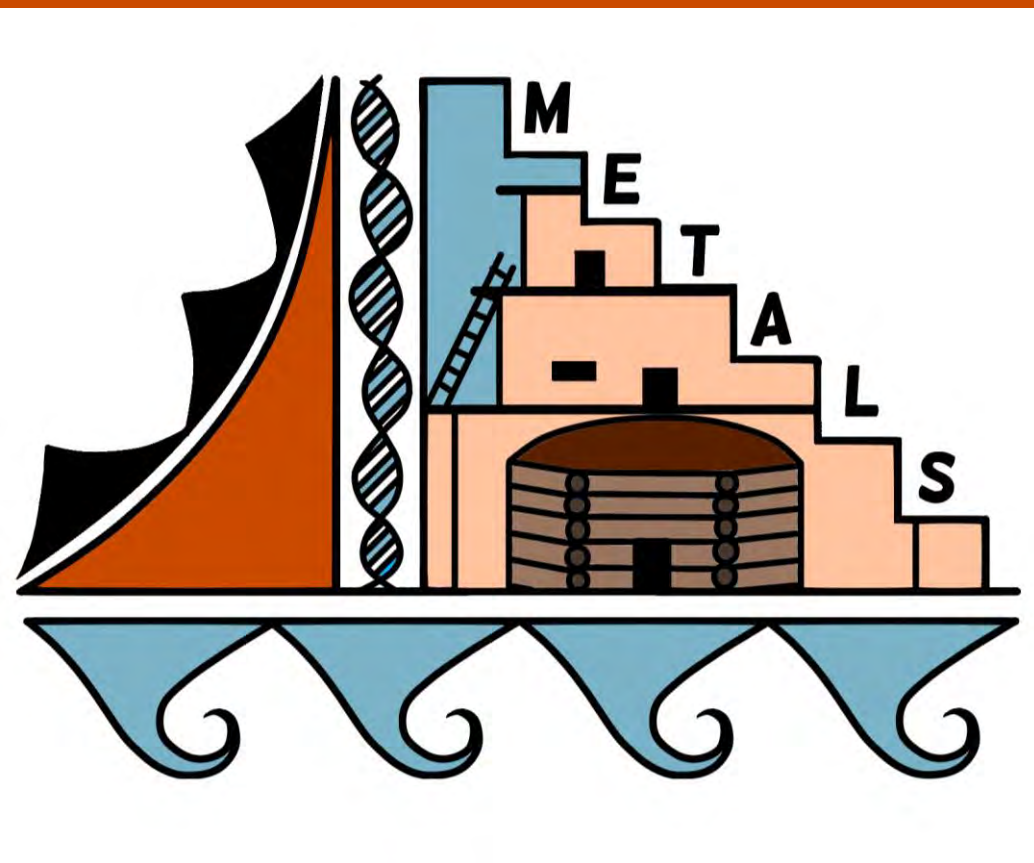




UNM METALS Superfund Center Overview :

Environmental Health Research on Uranium Mine Waste Exposures in the Southwest.

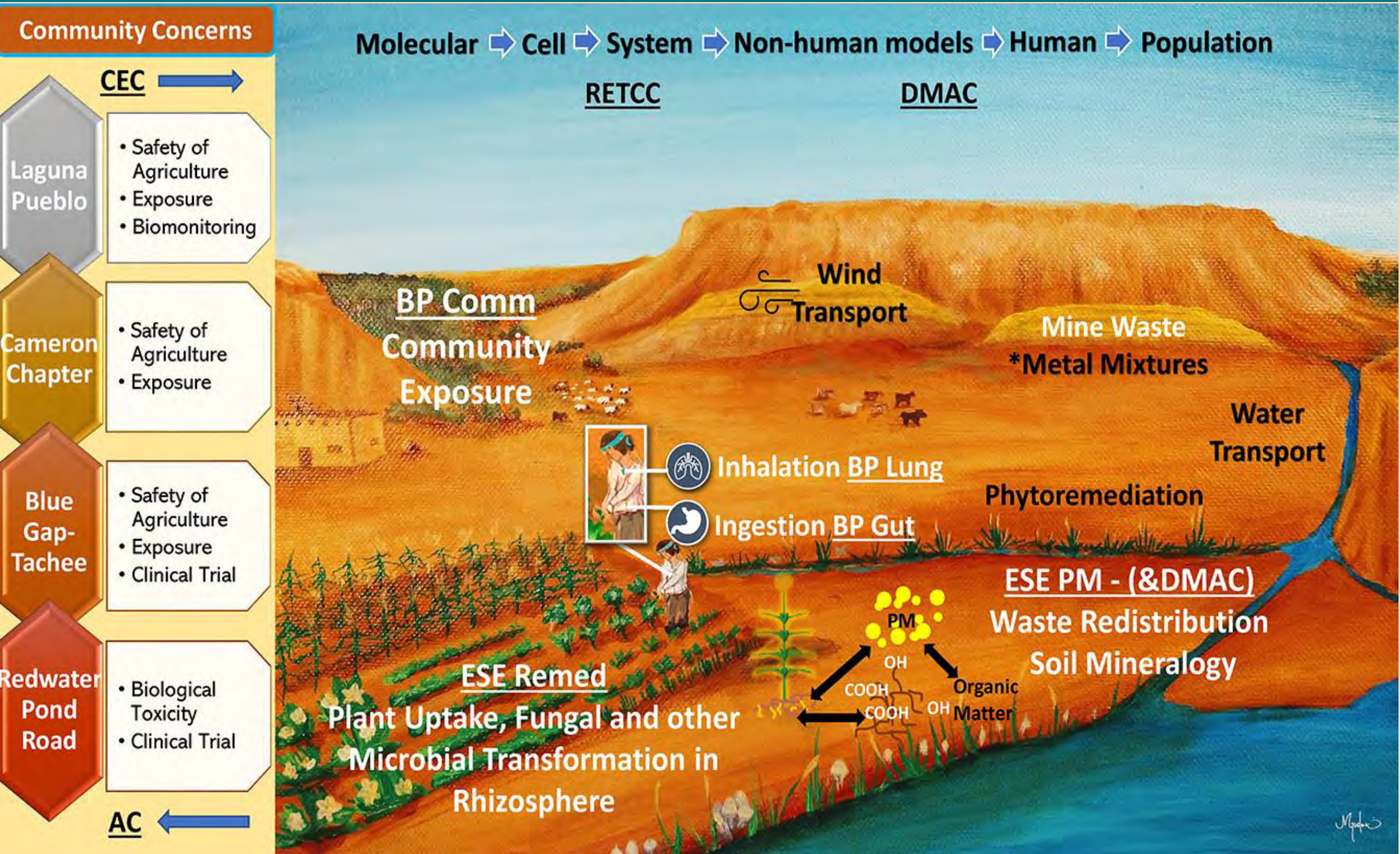
METALS Research Team,

Dr. Carolyn W. Roman, Dr. José M. Cerrato (Director)

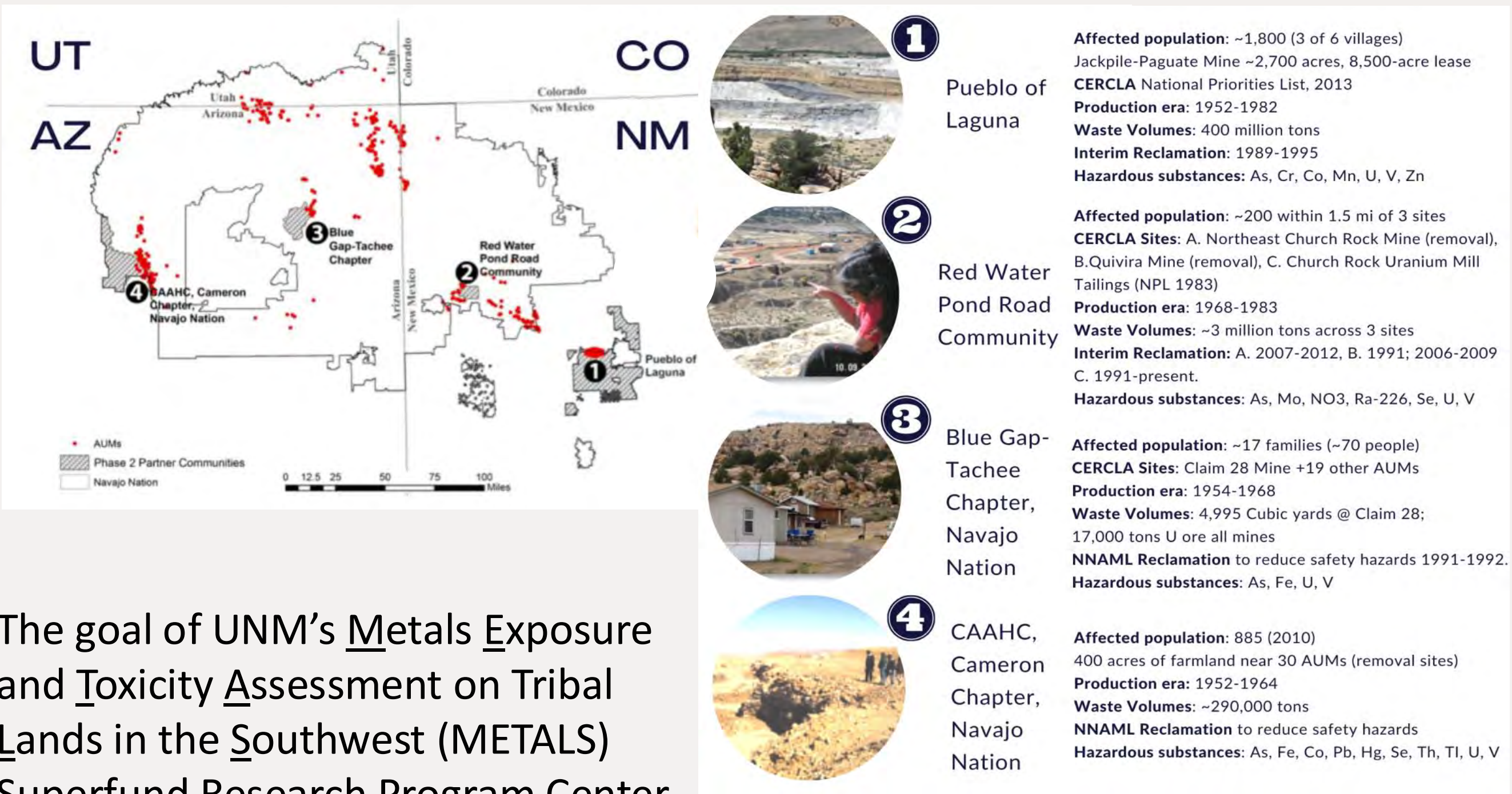
University of New Mexico Health Sciences Center, Community Environmental Health Program, College of Pharmacy, Albuquerque, NM



METALS Conceptual Model



Community Partners

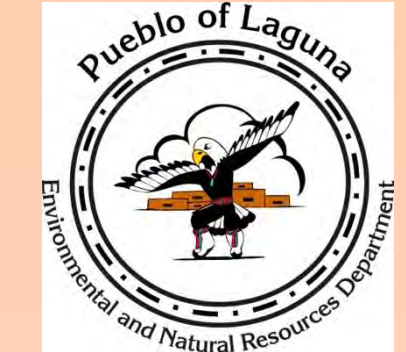


The goal of UNM's Metals Exposure and Toxicity Assessment on Tribal Lands in the Southwest (METALS) Superfund Research Program Center is to reduce risks to tribal populations impacted by metals mixtures from abandoned uranium mine (AUM) waste sites.

More than 4000 AUMs are documented in 15 western states that are home to the highest percentages of Native American population in the country.

- In our tribal cohorts and related laboratory studies we have
- 1) confirmed these exposures increase the risk of chronic diseases,
 - 2) demonstrated metal-specific additivity, synergy, and antagonism of AUM-specific metals in systemic and cellular mechanisms associated with these diseases, and
 - 3) identified potential toxicological interventions to protect affected populations.

Health Studies at the Pueblo of Laguna



In 2025 METALS has begun human health studies at the Pueblo of Laguna aimed to assess the impacts of exposure to uranium and mixed metals in a community living for decades near the Jackpile-Paguate Mine, once one of the world's largest open-pit uranium mines.



By the end of 2025, METALS has enrolled and collected surveys and biosamples from 115 participants from 6 different villages at the Pueblo of Laguna. METALS leads meet monthly with a Community Advisory Board (CAB) and our CEC team attends village meetings to update community members and get guidance on any protocol changes.



METALS Biological Projects (BPs):

1) BP Community – Led by Drs. Laurie Hudson and Debra MacKenzie
Goal: To understand the effects of uranium mine-associated metals on immune function and assess potential mechanism-based mitigation strategies.

BP Comm seeks to understand exposure-outcome relationships through study of mechanisms of cellular toxicity, DNA damage and repair processes, and metals-induced immune dysregulation. This project also examines how interventions, such as zinc supplementation, can mitigate health risk associated with metals exposure.

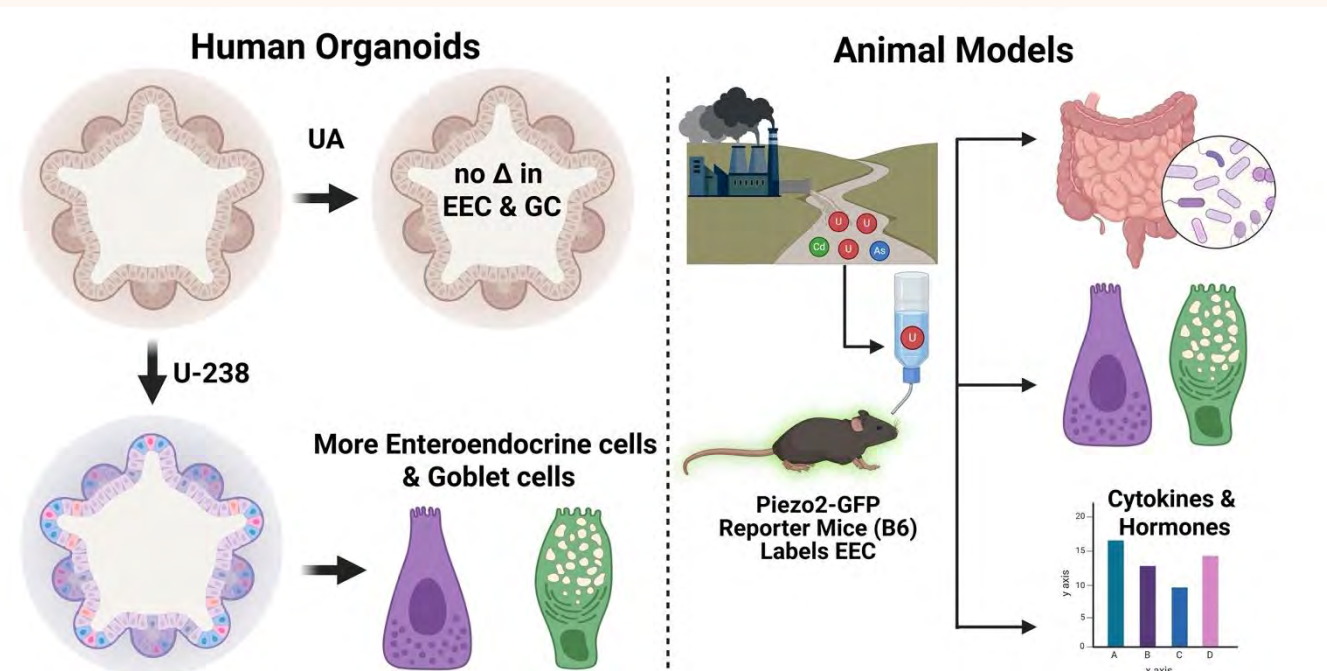
2) BP Lung – Led by Drs. Alicia Bolt, Sarah Blossom, and Katie Zychowski
Goal: Explore how inhaled metal-rich particulates drive immunological dysregulation and lead to lung and systemic autoimmunity.

This project investigates how exposure to uranium-bearing dust induces inflammation in the lungs and how the formation of neutrophil extracellular traps (NETs) contributes to immune dysregulation.

3) BP Gut - Led by Drs. Eliseo Castillo and Julie In

Goal: Explore cellular/molecular mechanisms behind the changes seen in the gut following mixed metals exposures.

Using cell models and organoids, in addition to human studies, BP Gut explores how metals exposure can induce injury like responses in gut cells and/or changes in gut metabolites and microbiota.



Environmental Science and Engineering (ESE) Projects:

1) ESE Particulate Matter (PM)

Led by Drs. Adrian Brearley and Eliane El Hayek

Goal: To study the distribution and bioavailability of uranium and co-occurring metals in soils and in air near abandoned uranium mines.

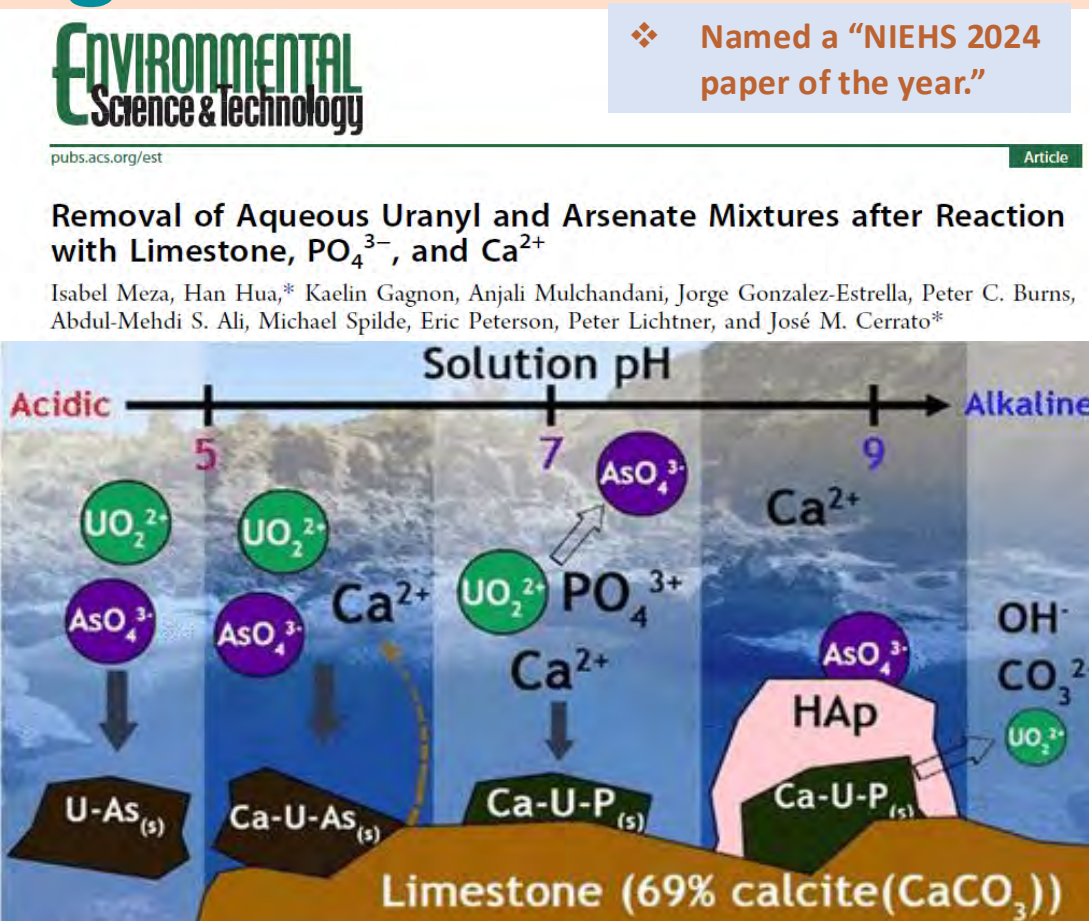
ESE PM investigates the composition and physical properties of mine wastes focusing on nanoparticle-sized fractions which pose an inhalation risk. These identify mechanisms and interactions that influence the bioavailability and transport of toxic metals.

2) ESE Remediation

Led by Drs. Jose Cerrato and Anjali Mulchandani

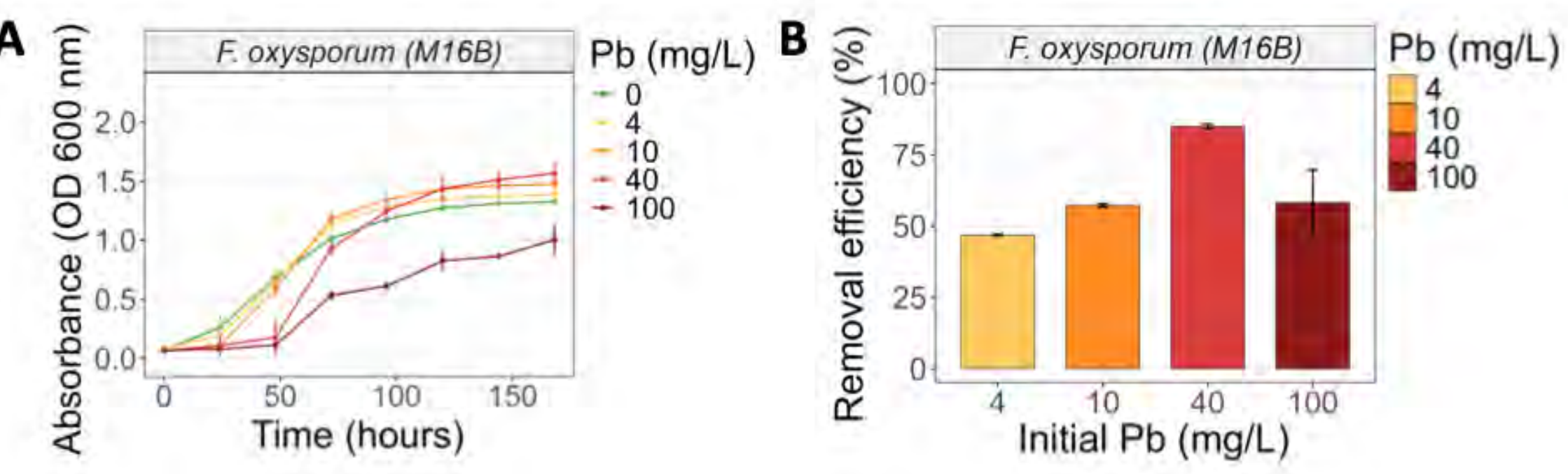
Goal: To investigate remediation of metals at AUM waste sites with naturally occurring solids and through bioremediation using plants and fungi.

This study has identified chemical conditions affecting mobilization of uranium and arsenic in water as well as in the roots and shoots of plants and continues making progress using natural minerals for immobilization of U and As.



Sourcing Plants and Fungi from the Jackpile-Paguate Mine

In collaboration with the Pueblo of Laguna, trainees collect and evaluate candidates for bioremediation of metals. Sunflowers and seeds have been collected from Paguate to find out where sunflowers store metals. Plants are then grown in the research greenhouse in soils with heavy metals to further study their uptake. Fungi are collected to study their tolerance to metals mixtures and plant-fungi interactions influencing metals uptake.



Mine-sourced fungi, *Fusarium oxysporum*, is less sensitive to Pb exposure (growth in increasing Pb,) and shows high Pb removal capabilities (80% removal at 40 mg/L of Pb, Panel B).



Fungal tolerance to lead (Pb) is concentration and provenance dependent

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Contextualization

Jackpile-Paguate Uranium Mine Superfund Site



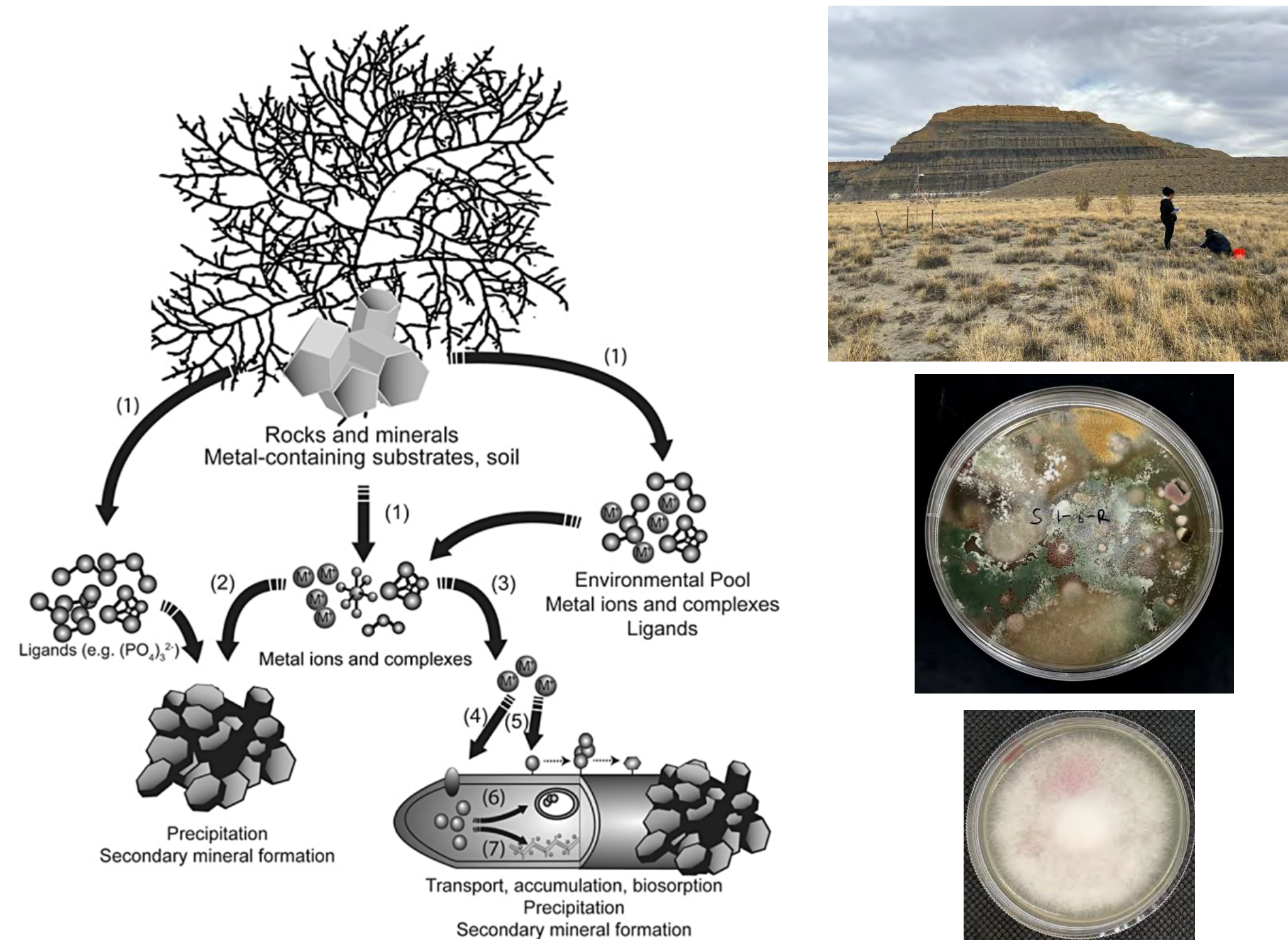
- One of the world's largest open-pit uranium mine
- 30 years of operation: 1952 to 1982 - No longer in operation
- Clean-up plan: 1989 - 1995

- Pueblo of Laguna** (40 miles west of Albuquerque)
 - Three villages edge the mine
- Mining legacy directly impacts the Pueblo of Laguna
 - Aggravating risk factors in exposure-toxicity
- Co-occurrence of metal mixtures: Uranium (U), Arsenic (As), **Lead (Pb)**
 - non-essential element with high toxicity and long-term persistence in the environment.

Microbial remediation might be an economic, sustainable and culturally responsive alternative.

Fungi-metal interactions

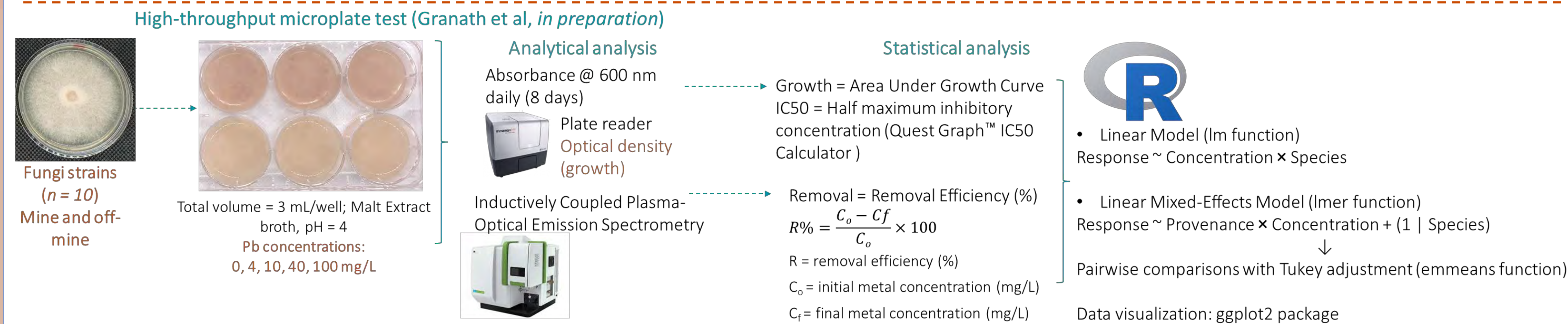
Fungi are microorganism that naturally occur in mine sites and interact with metals, removing them from the environment or transforming metals into less toxic forms.



It is of utmost importance to understand metal tolerance in fungi to assess removal capabilities for environmental applications.

Hypothesis & Methods

Hypothesis: Fungi's ability to grow in the presence of Pb and remove the metal from aqueous solution is affected by 1. Pb concentration, and 2. Fungi's provenance (mine vs. off-mine)



Results: Growth and Removal of Pb in increasing concentrations

Figure 1A. Pb tolerance in increasing concentration varied by species with significant decrease in growth at 100 mg/L ($p < 0.001$, $R^2 = 0.96$).

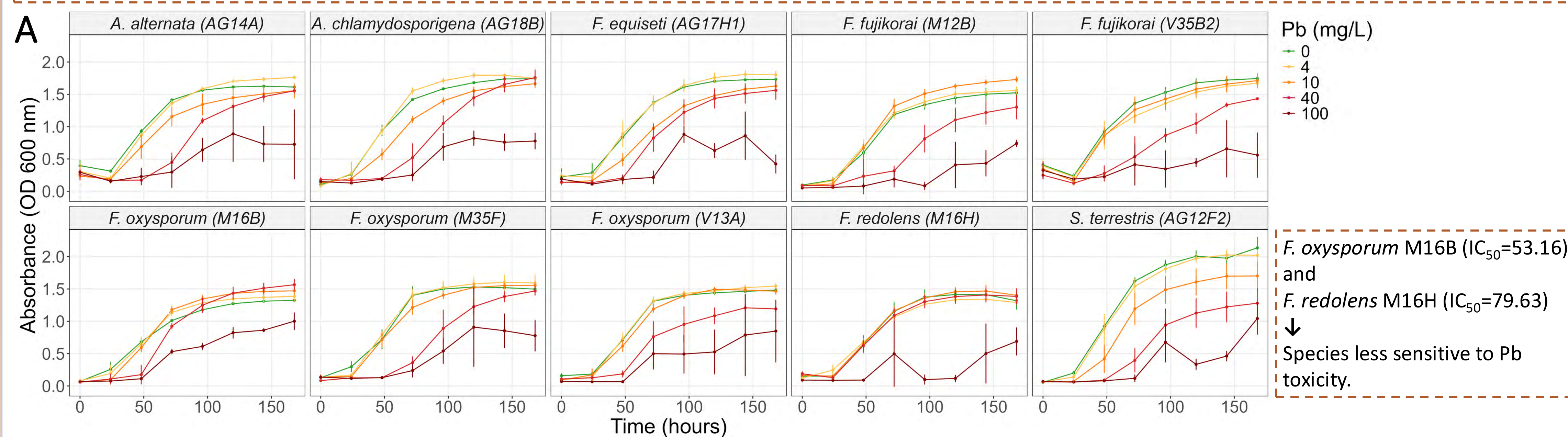
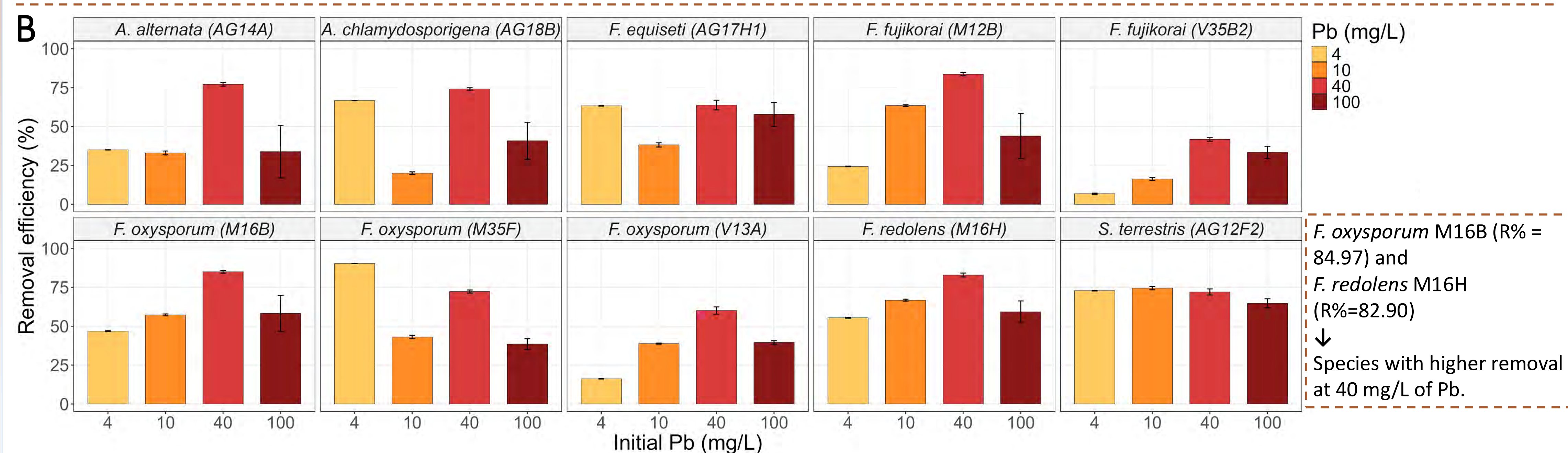


Figure 1B. Pb removal efficiency varied by species and peaked at 40 mg/L for most of the species ($p < 0.001$, $R^2 = 0.95$).



Results: Mine vs. off-mine

Figure 2A. Provenance did not affect fungal growth ($p=0.3434$), except at 40 mg/L, where fungi from mine outgrew off-mine fungi by 23.04% ($p<0.05$).

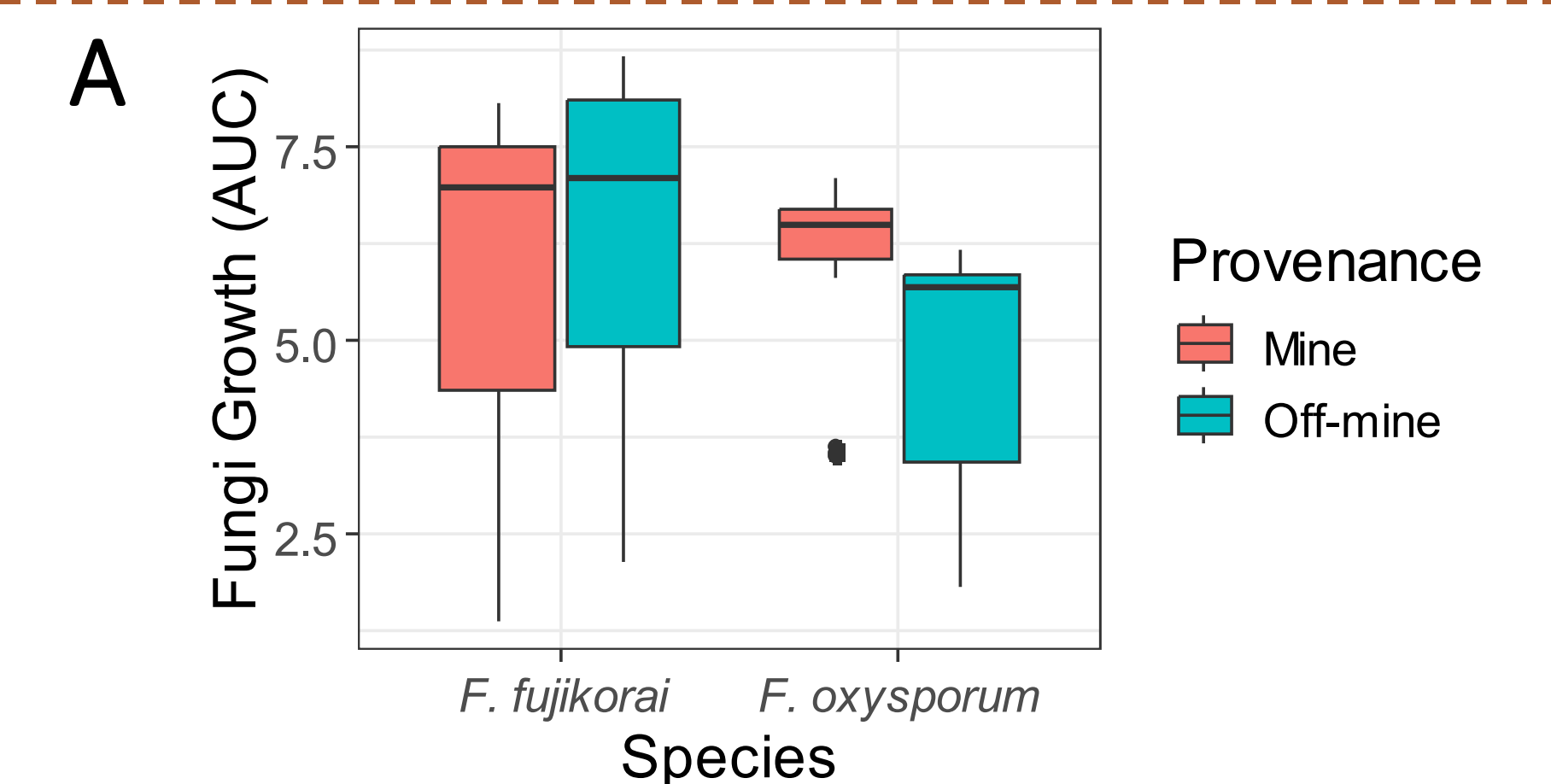
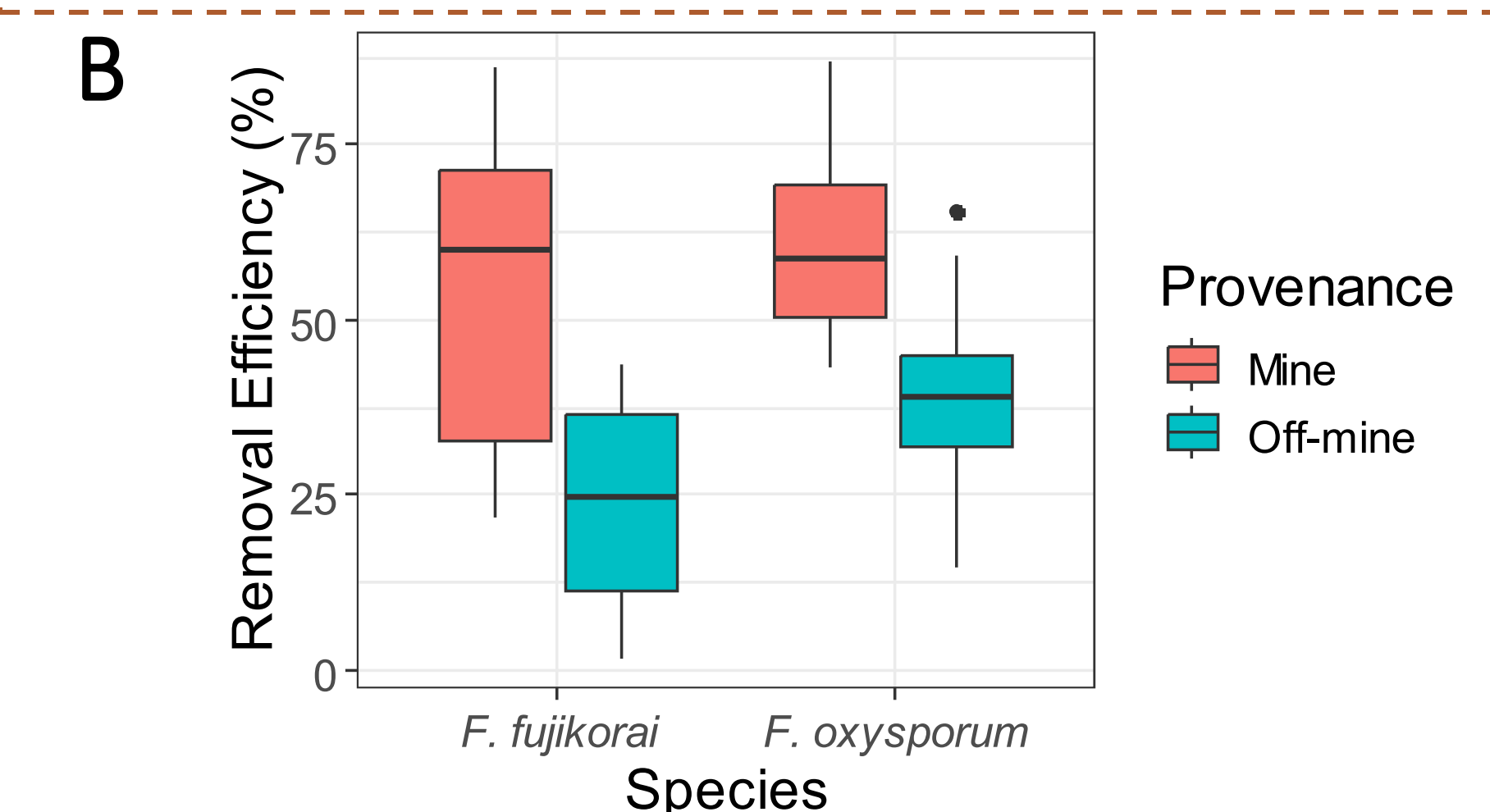


Figure 2B. Provenance affects removal efficiency of Pb by fungi ($p<0.01$), where fungi from mine significantly outperformed off-mine in Pb removal.



Conclusions & Ongoing Work

- Both **mine-sourced** *F. oxysporum* and *F. redolens* were identified as the most effective candidates: less sensitive to Pb and higher removal efficiency.
- Ongoing Work:**
 - STEM-EDX: cell micromorphology to detect Pb in fungi cells.
 - FTIR and Raman Spectroscopy: to determine functional groups on the fungal cell surface involved in metal binding.

Our findings highlight the potential of fungi naturally adapted to metal-contaminated environments as a promising and environmentally relevant approach for remediating polluted sites.

Background

Background

- There are over 160,000 abandoned uranium mines (AUMS) throughout the Western United States.
- Heavy metals from AUMS leach into the environment leading to increased exposure in the surrounding communities.
- Environmental heavy metals are ingested or inhaled leading to early exposure in the gastrointestinal tract.
- The mucus layer of the gut, produced by goblet cells, is the first line of defense between the host and ingested toxins and pathogens.
- How does a heavy metal such as uranium impact the gastrointestinal tract with regards to the mucus layer dynamics and goblet cell physiology?

Objective: Using human derived intestinal organoids (HIOs), we aim to delineate how uranium-bearing dust (UBD) impacts the gut colonic mucus layer and goblet cell physiology.

Methods

UBD was collected from the Pueblo of Laguna which is adjacent to the Jackpile Paguate Uranium mine in New Mexico. Scanning transmission electron microscopy (STEM) and STEM energy dispersive X-ray spectroscopy (Eds) maps were used to characterize UBD (Fig. 1).

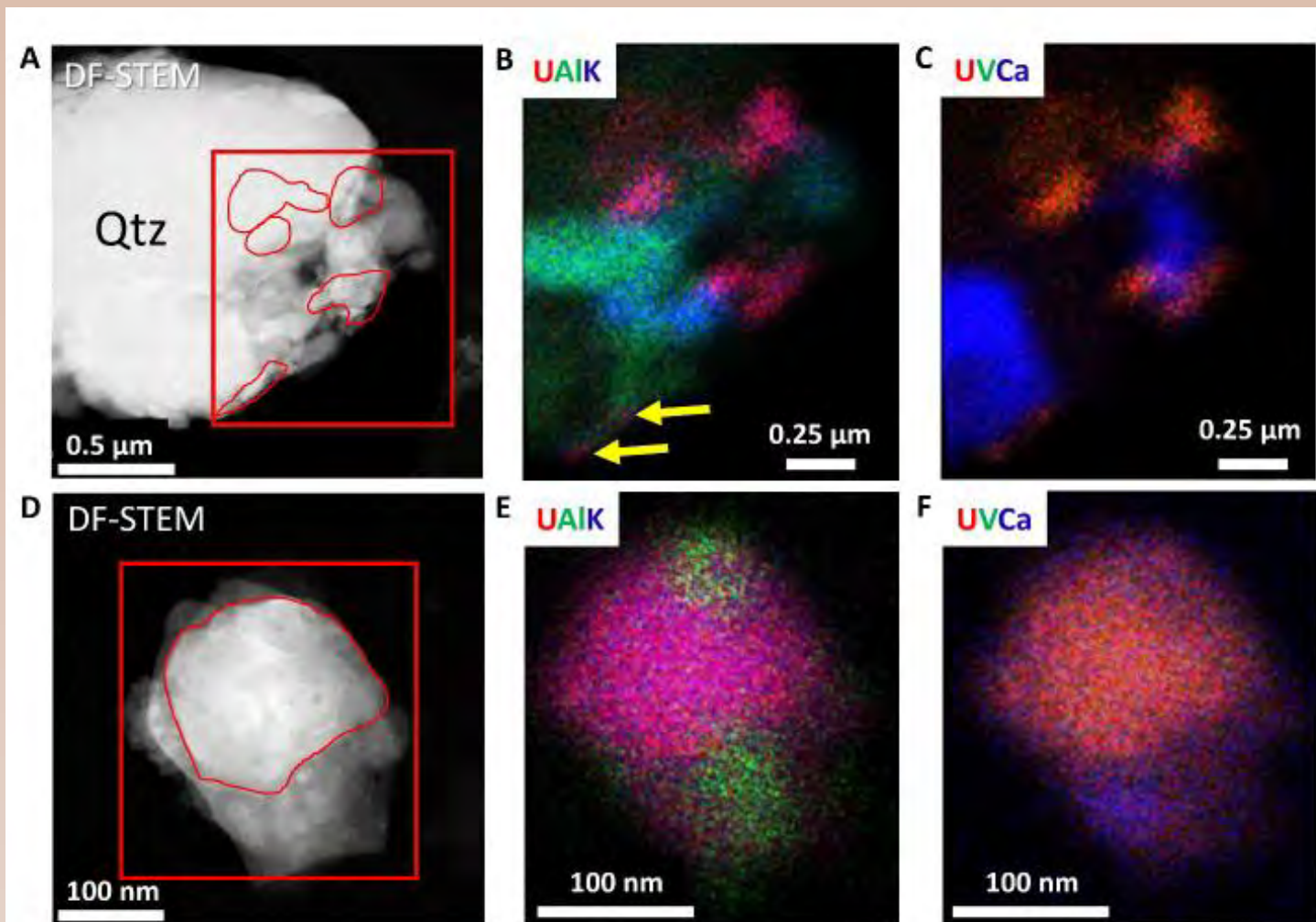


Figure. 1 Quartz grain (Qtz) with red box outlining U-bearing grains identified with STEM and EDs. U, uranium; Al, aluminum; K potassium; V, vanadium; and CA, calcium were identified in the dust samples. U-bearing grains were small and 50 nm or less. The abundance of u-bearing grains was ~0.01%.

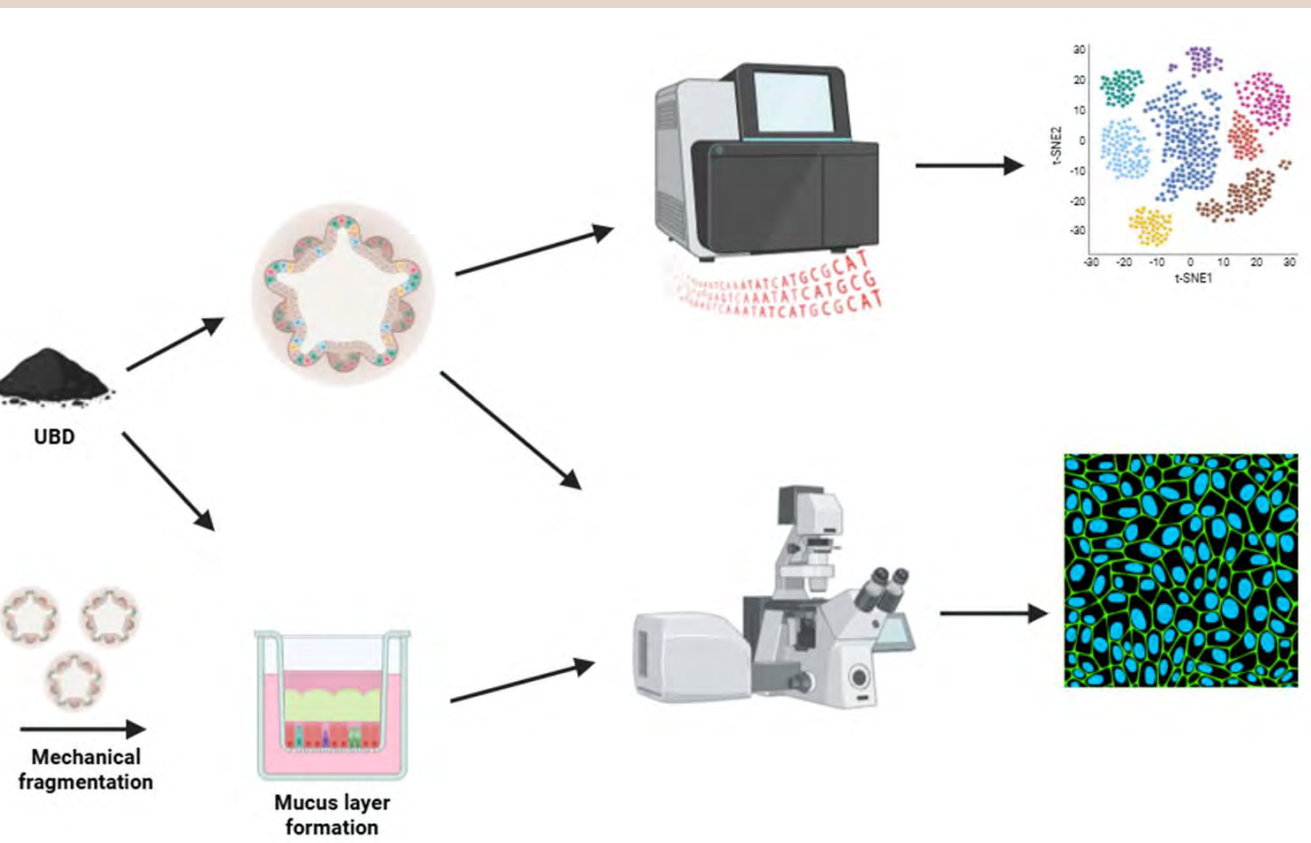


Figure 2: Colonic HIOs were exposed to UBD for 24hrs. HIOs were digested for scRNA-seq. Seq analysis was validated with advanced microscopy and immunoblotting. To assess secreted mucus layer, HIOs are fragmented and plated as monolayers on Transwells. *Image created in Biorender*

Results

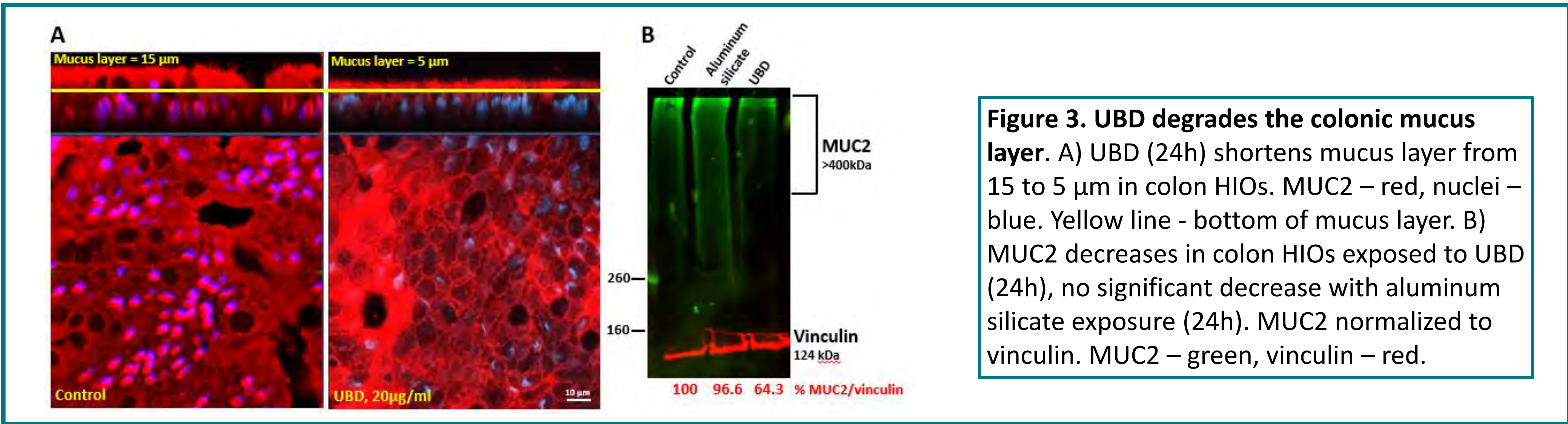


Figure 3. UBD degrades the colonic mucus layer. A) UBD (24h) shortens mucus layer from 15 to 5 μ m in colon HIOs. MUC2 – red, nuclei – blue. Yellow line - bottom of mucus layer. B) MUC2 decreases in colon HIOs exposed to UBD (24h), no significant decrease with aluminum silicate exposure (24h). MUC2 normalized to vinculin. MUC2 – green, vinculin – red.

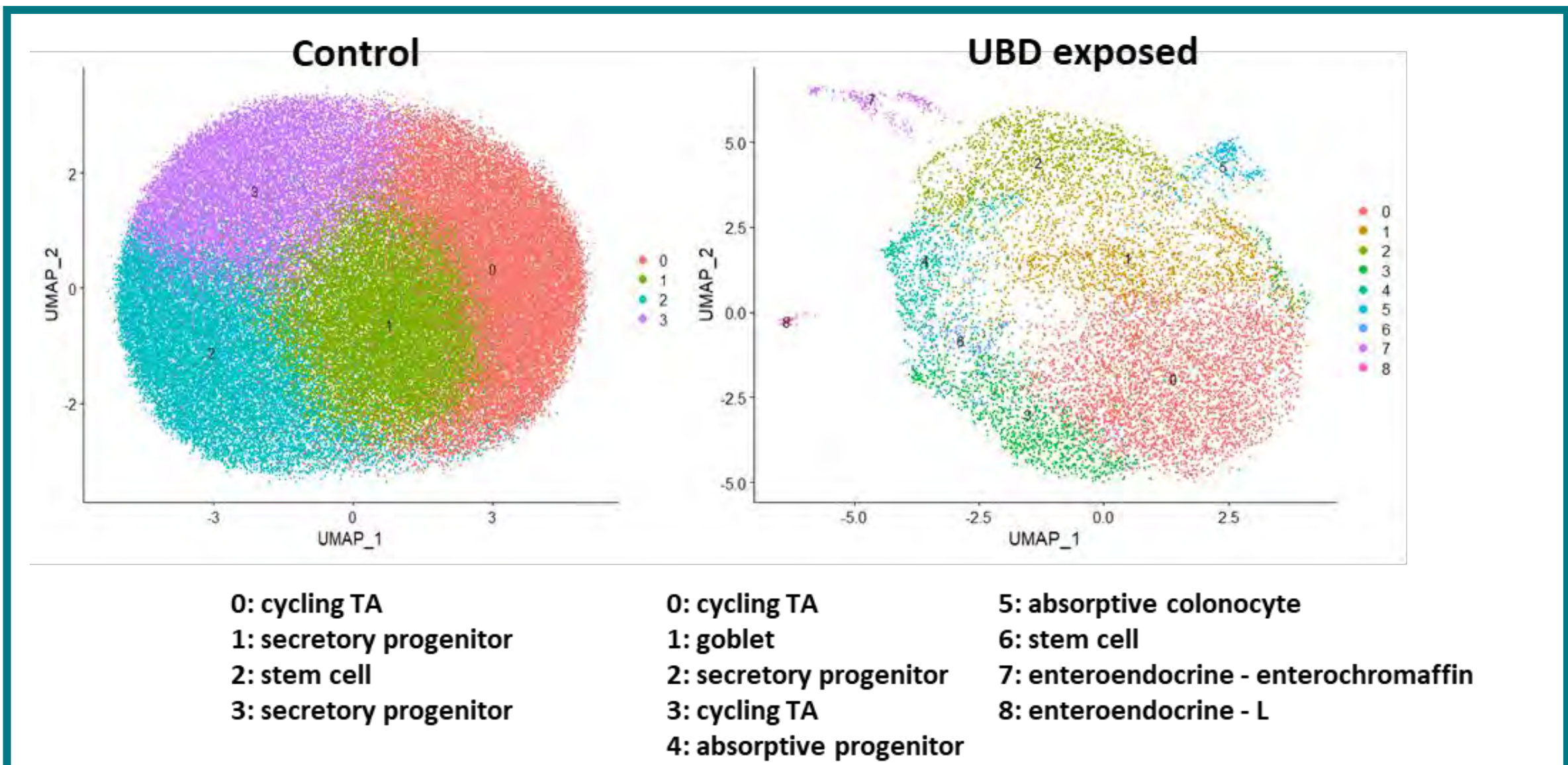


Figure 4. UBD exposure induces differentiation to the secretory lineage in colon HIOs. Representative UMAP plots of control (88,085 cells) and UBD exposed (10,374 cells) HIOs reveal a shift into the secretory lineage – goblet cells.

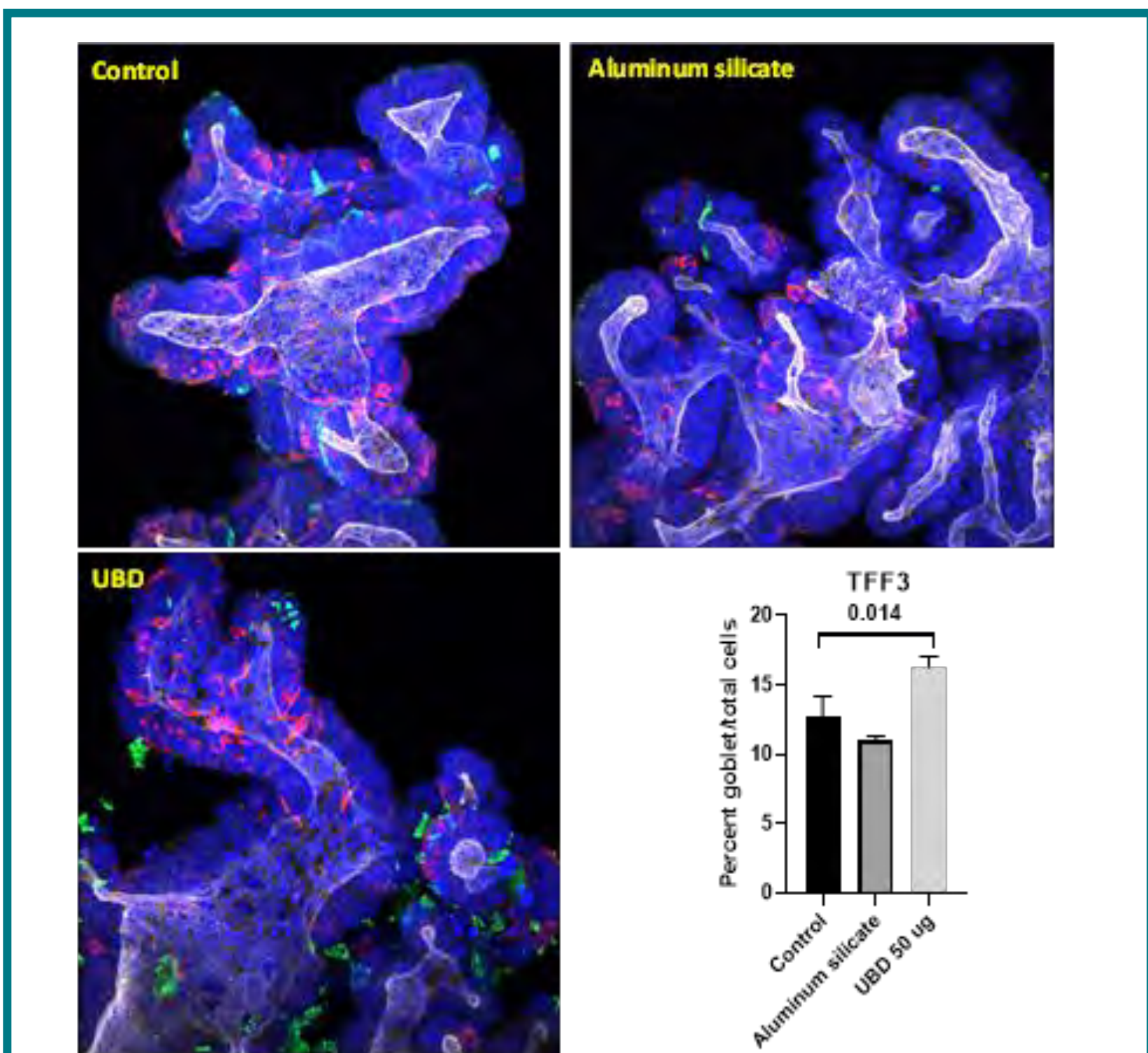


Figure 6. Colon HIOs exposed to UBD (24h) had increased numbers of goblet cells (TFF3). Aluminum silicate had no effect. Mean $\pm$ SEM. Phalloidin – white, TFF3 – red, nuclei – blue

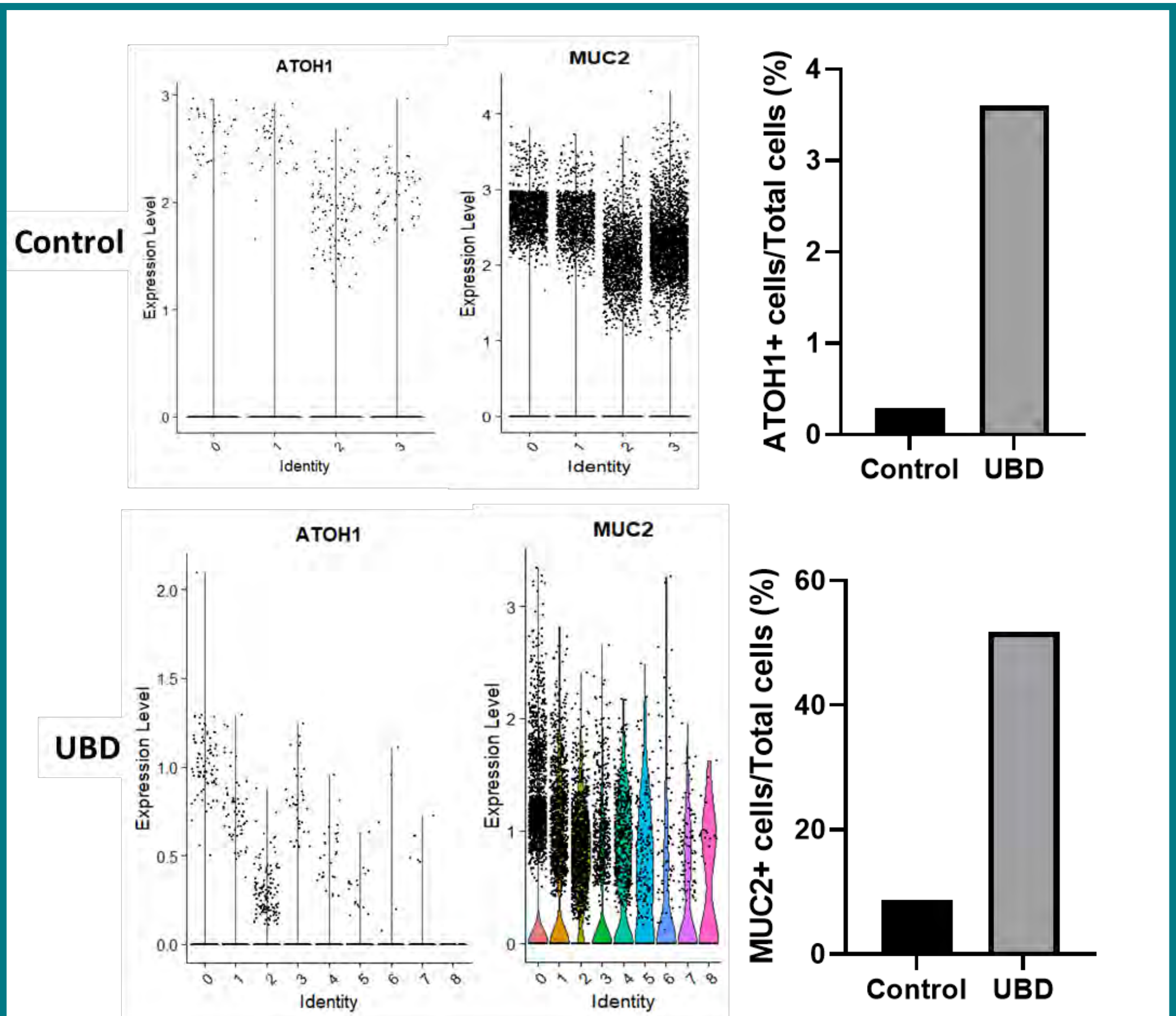


Figure 5. UBD exposure induces differentiation to the secretory lineage in colon HIOs. Representative UMAP plots of control (88,085 cells) and UBD exposed (10,374 cells) HIOs reveal a shift into the secretory lineage – goblet cells.

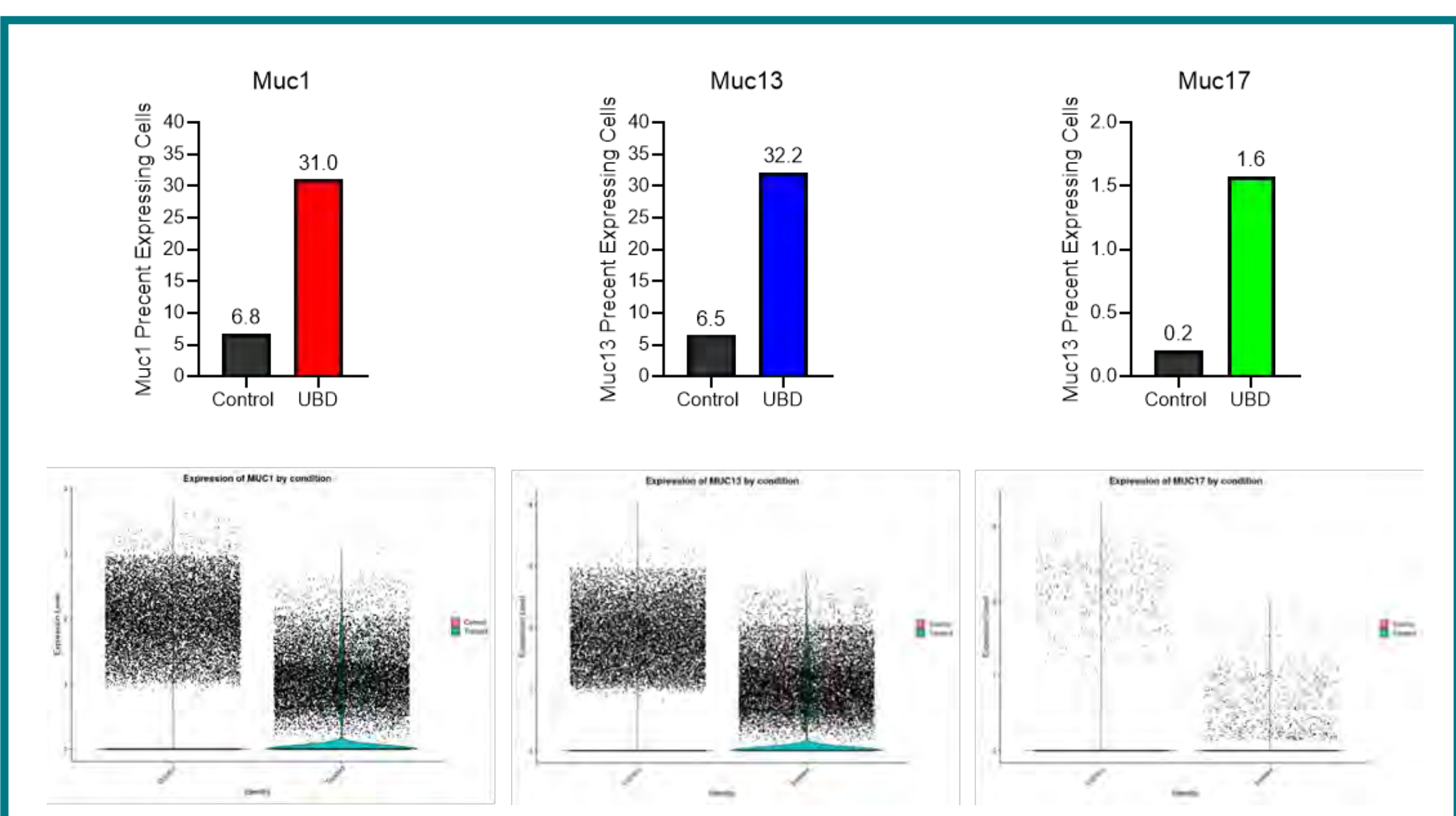


Figure 7. Bar graphs show UBD treatment increases in adherent mucins Muc1, Muc3, and Muc17 percent expressing cells. The violin plots of transcript expression level per cluster with control on the left and treatment on the right.

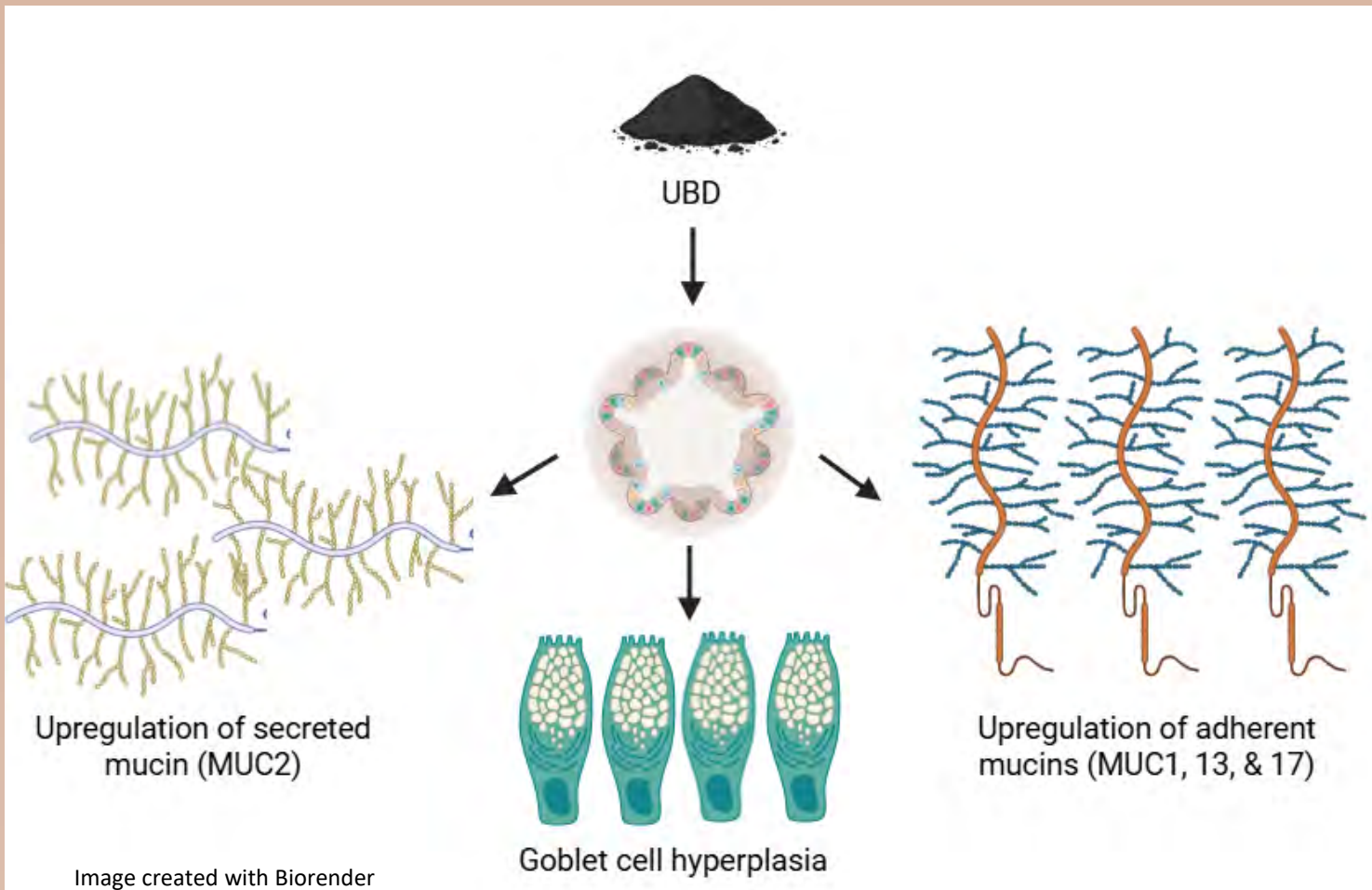
Results and Conclusions

Results:

- UBD (24hr) degrades the mucus layer in HIO monolayers (Fig. 3)
- UBD treatment leads to expansion of the secretory lineage (Fig. 4)
- UBD treatment leads to hyperplasia of goblet cells and upregulation of Muc2, the primary mucin protein (Fig. 5 and Fig. 6)
- UBD exposure also led to upregulation in the number of cells expressing adherent mucins Muc1, Muc13, and Muc17 (Fig. 7)

Conclusion:

Our results indicate that UBD exposure degrades the gastrointestinal mucus layer resulting in GC hyperplasia and upregulation of cells expressing mucins to restore the barrier integrity.



Future Directions

- Examine time kinetics of mucus layer replenishing following UBD exposure
- Assess changes in goblet cell sub-populations (intercrypt, sentinel, surface) as well as differing mucus types from the deep crypt vs the surface
- Determine alterations in autophagy and SNARE proteins in mucus secretion following UBD exposure
- Determine role(s) of adherent mucins in MUC2+ mucus layer secretion and response to UBD and other heavy metals

Acknowledgements

This work was supported by grants from the National Institutes of Health: R01ES034400 and P42ES025589. Technical assistance for single-cell sequencing was provided by the UNM Comprehensive Cancer Center (UNMCC) Analytical and Translational Genomics Core. Microscopy images were obtained at the UNMCCC Fluorescence Microscopy Core. Additional support is provided from UNM College of Pharmacy and UNMCCC. Many thanks to UNM SRP, POL ENRD, and all members of the In Lab.

Introduction

Chronic exposure to uranium (U)-rich environments is a public health crisis, yet the molecular mechanisms driving these effects are not well understood. Extracellular vesicles (EVs) are membrane-bound particles that relay signals between cells and modulate cellular function. To investigate whether U-mining tenure is associated with specific changes in serum-derived EV proteomic and plasma cytokine profiles in former Uminers, and to evaluate the potential of EV-derived proteins as sensitive biomarkers of chronic U-exposure compared to canonical cytokines. In a second set of experiments, we compared macrophage cellular functional assays in macrophages exposed to control, coal (Colorado Coal) and U-dust (St. Anthony Mine). Results: Eight small-EV and four large-EV proteins were significantly correlated with mining tenure after adjusting for age. In addition, U-dust exposure on macrophages resulted in the greatest functional changes in bioenergetics and phagocytosis endpoints. Future studies will evaluate the role of EVs, post dust exposure on macrophage function.

Methods

Serum and plasma samples were collected from 39 former U-miners. Small and large EVs were isolated via differential ultracentrifugation and characterized using nanoparticle tracking analysis and western blotting. EV proteomes were analyzed by liquid chromatography-tandem mass spectrometry, while plasma cytokines were quantified using multiplex immunoassays. Associations with mining tenure were assessed using age-adjusted linear regression, and pathway enrichment analyses were performed on significant EV proteins. In the secondary set of experiments, macrophages were cultured and exposed to relevant dusts (50ug/mL). Bioenergetics and phagocytosis assays were subsequently performed.

Results

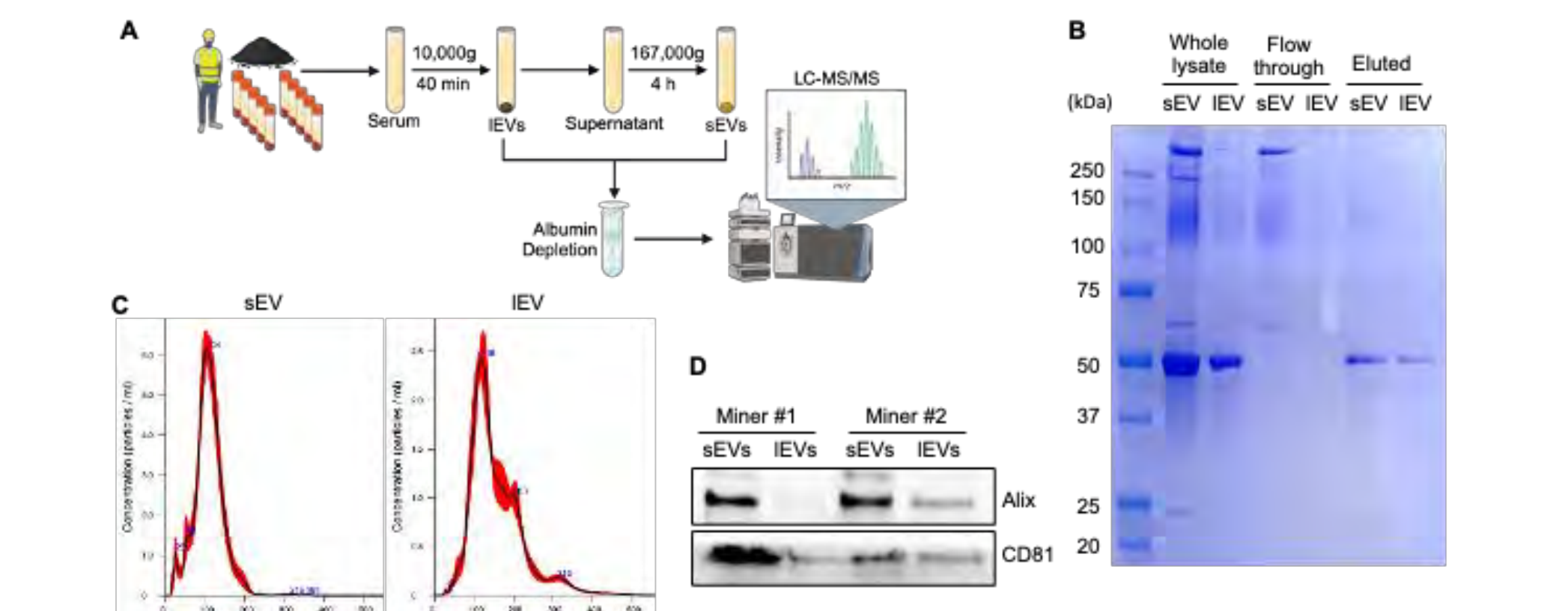


Figure 1. Isolation and characterization of extracellular vesicles (EVs) from miners' serum. (A) Schematic workflow for isolation of small extracellular vesicles (S-EV) and large extracellular vesicles (L-EVs) from miners' serum, followed by proteomic analysis using liquid chromatography-tandem mass spectrometry (LC-MS/MS). (B) SDS-PAGE analysis showing efficiency of albumin depletion, with reduced band intensity in depleted samples compared to untreated controls. (C) Nanoparticle tracking analysis demonstrating size distribution and concentration of isolated vesicles. S-EV range primarily from 30–150 nm; L-EVs show broader distribution from 100–400 nm. (D) Western blot confirmation of EV-specific markers Alix and CD81 in sEV and L-EV samples, validating successful EV enrichment (adapted from Oh et al. 2025).

Marker	Abbreviation	Estimate (Log2 FC)	Confidence		p-value
			Interval (Low)	Interval (High)	
Complement C1r subcomponent	C1R	-1.146	-2.057	-0.236	0.016
Vitamin K-dependent protein S	Protein S	-1.073	-1.940	-0.205	0.017
Serum amyloid A-1 protein	SAA1	-0.939	-1.719	-0.159	0.020
Immunoglobulin lambda constant 3	IGLC3	0.836	0.095	1.577	0.028
Immunoglobulin lambda-like polypeptide 1	IGLL1	-0.679	-1.311	-0.047	0.036
Leucine-rich alpha-2-glycoprotein	LRG1	-0.987	-1.908	-0.066	0.037
Fibrinogen alpha chain	FGA	-1.491	-2.892	-0.090	0.038
Lumican	LUM	-0.696	-1.362	-0.030	0.041

Table 1. Candidate proteins in small extracellular vesicles (S-EV) correlated with uranium mining tenure *Protein Abbreviations:* Official protein abbreviations were used for clarity and consistency in reporting. *Estimate:* Regression coefficients (log2 fold change) adjusted for age, with mining tenure dichotomized (10+ years vs. 1–9 years). *Confidence Interval:* 95% confidence bounds for the regression estimate. *p-value:* Statistical significance threshold at $p \leq 0.05$. (adapted from Oh et al. 2025)

Marker	Abbreviation	Estimate (Log2 FC)	Confidence Interval (Low)	Confidence Interval (High)	p-value
Immunoglobulin heavy variable 3-73	IGHV3-73	0.606	0.064	1.148	0.030
Complement component C9	C9	1.381	0.082	2.681	0.038
Plasma kallikrein	PK	-0.901	-1.787	-0.015	0.047
Immunoglobulin kappa variable 1-17	IGKV1-17	-0.625	-1.242	-0.009	0.047

Table 2. Candidate proteins in large extracellular vesicles (L-EVs) correlated with uranium mining tenure *Protein Abbreviations:* Official protein abbreviations were used for clarity and consistency in reporting. *Estimate:* Regression coefficients (log2 fold change) adjusted for age, with mining tenure dichotomized (10+ years vs. 1–9 years). *Confidence Interval:* 95% confidence bounds for the regression estimate. *p-value:* Statistical significance threshold at $p \leq 0.05$. (adapted from Oh et al. 2025)

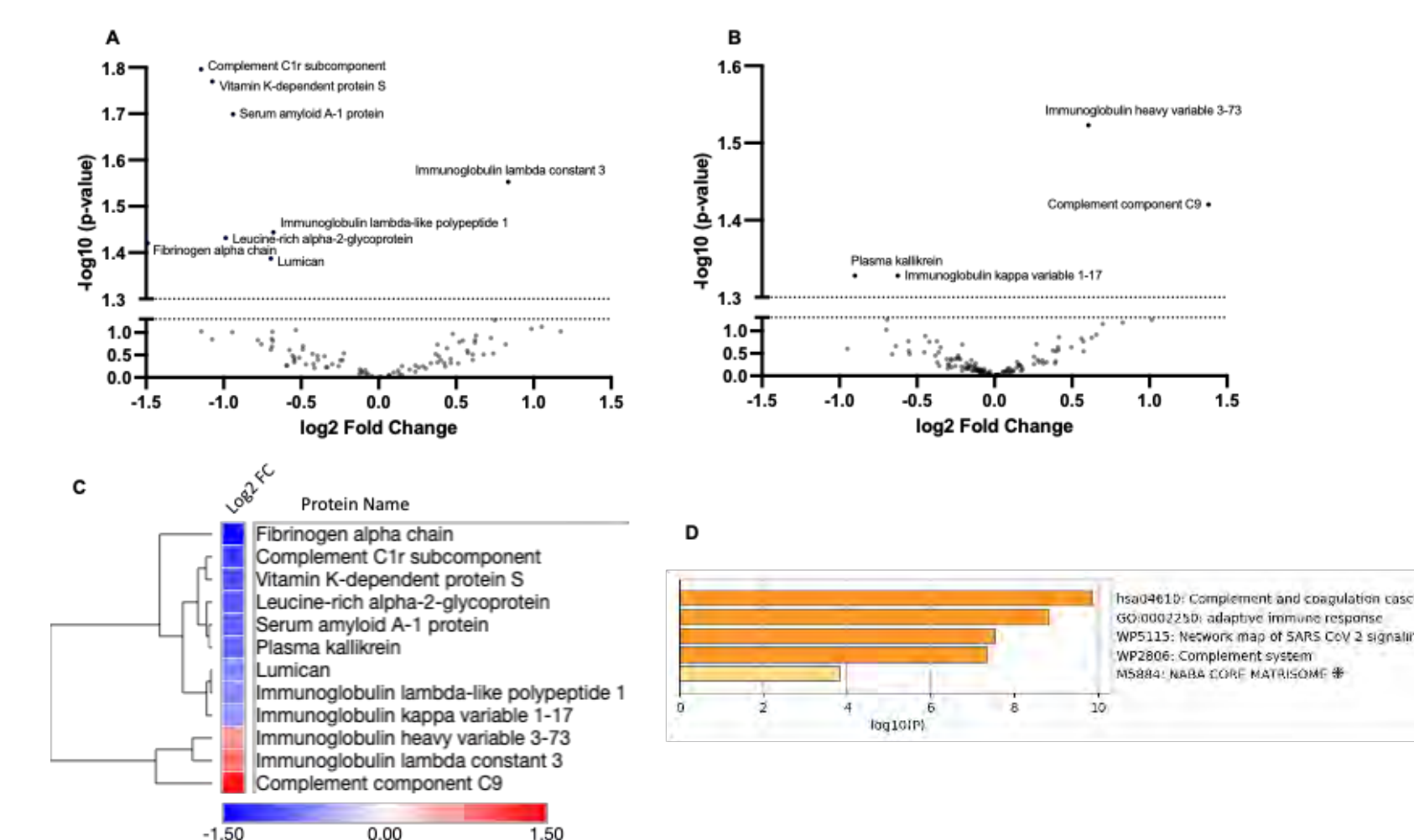


Figure 2. EV-associated proteomic and pathway signatures stratify uranium miners by occupational tenure. (A,B) Volcano plots of tenure-associated proteins in (A) S-EV and (B) L-EVs. Dashed line indicates $p = 0.05$ threshold. Red: positive correlation (estimate > 0); blue: negative correlation (estimate < 0). Key proteins with significant associations ($p \leq 0.05$) are labeled. (C) Heatmap of significantly correlated proteins ($p \leq 0.05$) from both S-EV and L-EVs. Color scale: red = positive correlation, blue = negative correlation. Dendrogram reflects Euclidean distance clustering of protein expression patterns across samples. (D) Top functional pathways identified by Metascape enrichment analysis. Bar height represents $-\log_{10}(q\text{-value})$; colors indicate statistical significance for pathway categories. SARS-CoV-2 Signaling and NABA CORE MATRIAOME pathways are highlighted with * (adapted from Oh et al. 2025).

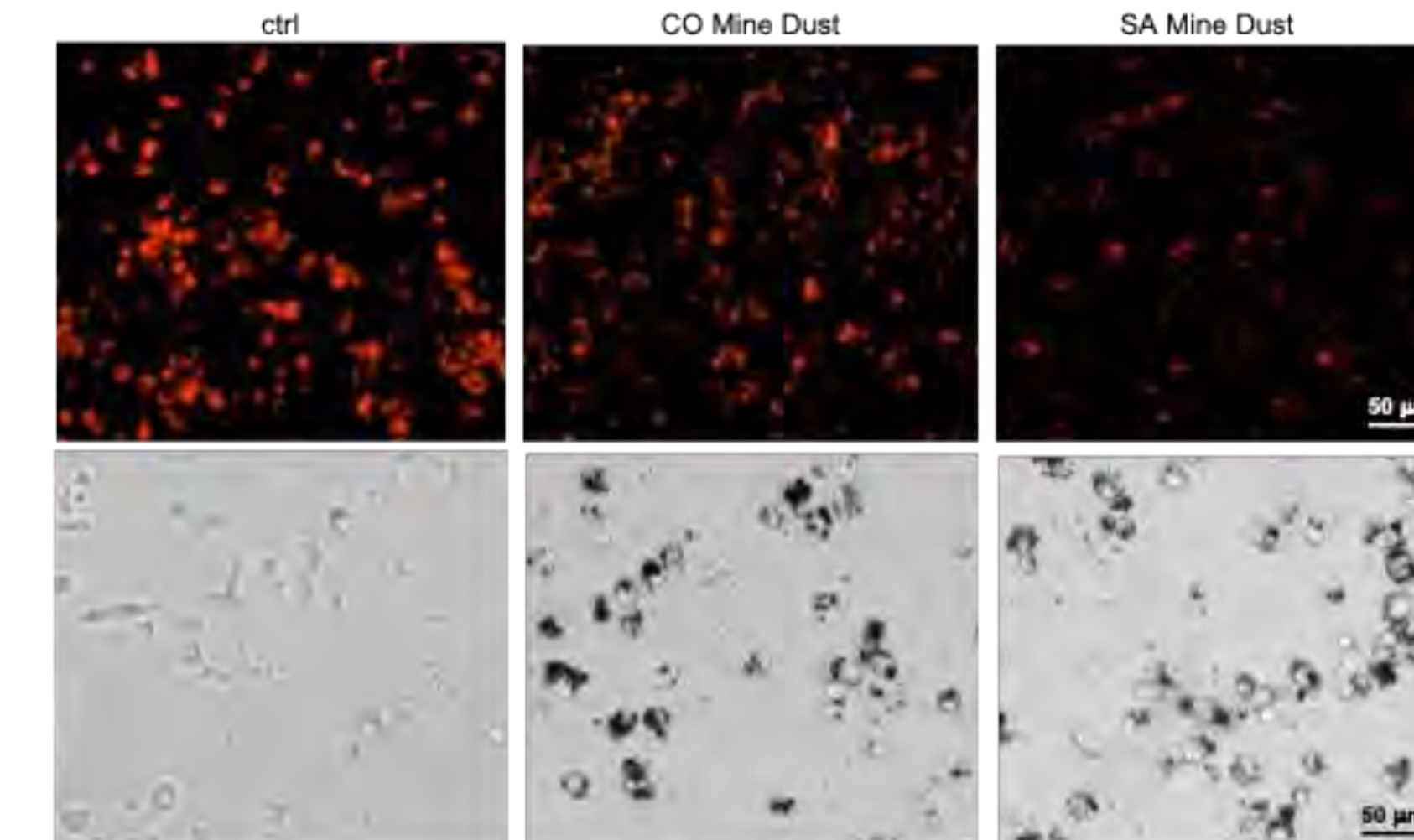


Figure 3. Fluorescent and light microscopy. Vehicle control, Colorado mine-dust and St. Anthony mine-dust (U-mine) treated differentiated macrophages for 4h. THP-1 macrophages were used for these dust treatments (50 µg/ml).

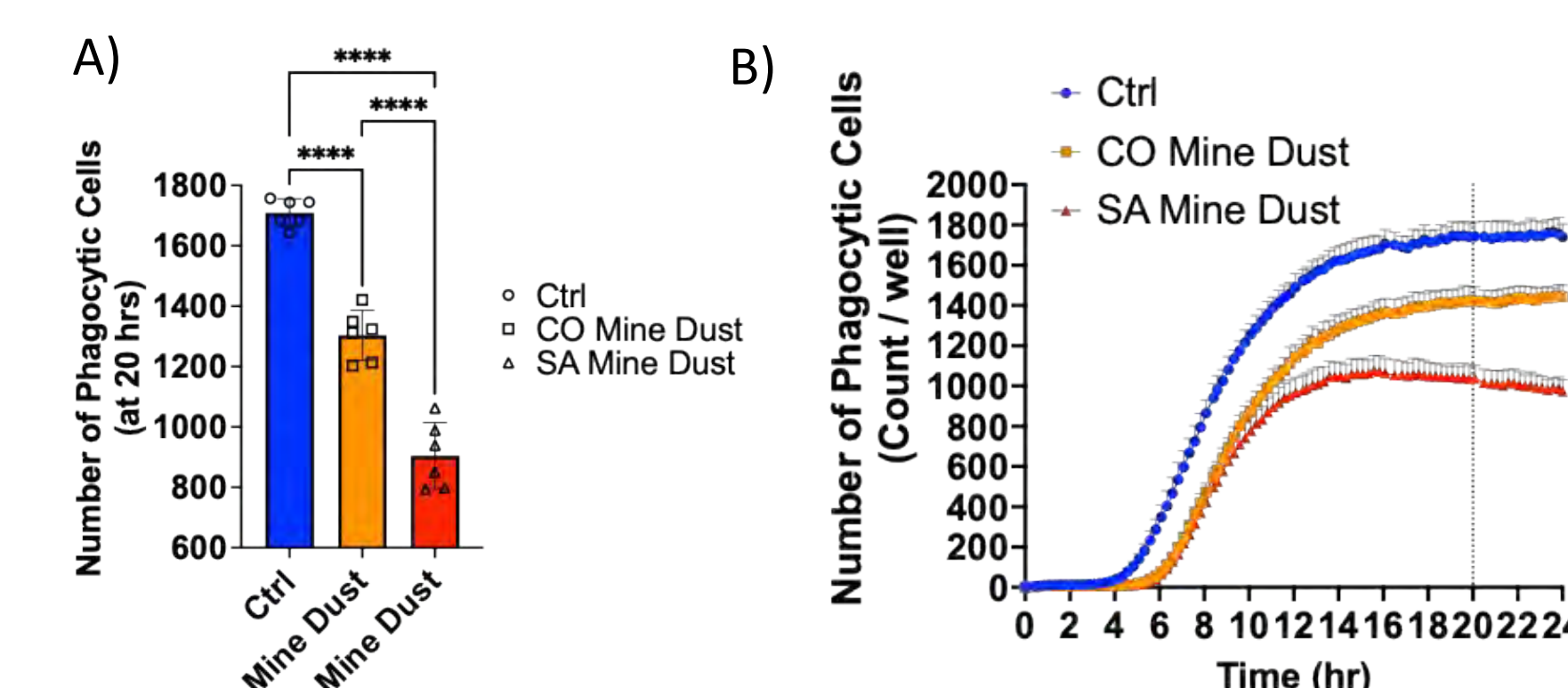


Figure 4. Phagocytosis assay in macrophages treated with mine-dust. THP-1 macrophage cells were treated with 50 µg/ml dust for 4h and phagocytosis was monitored according to a previously-established protocol. A) number of phagocytic cells according to each treatment B) phagocytosis over time.

Results

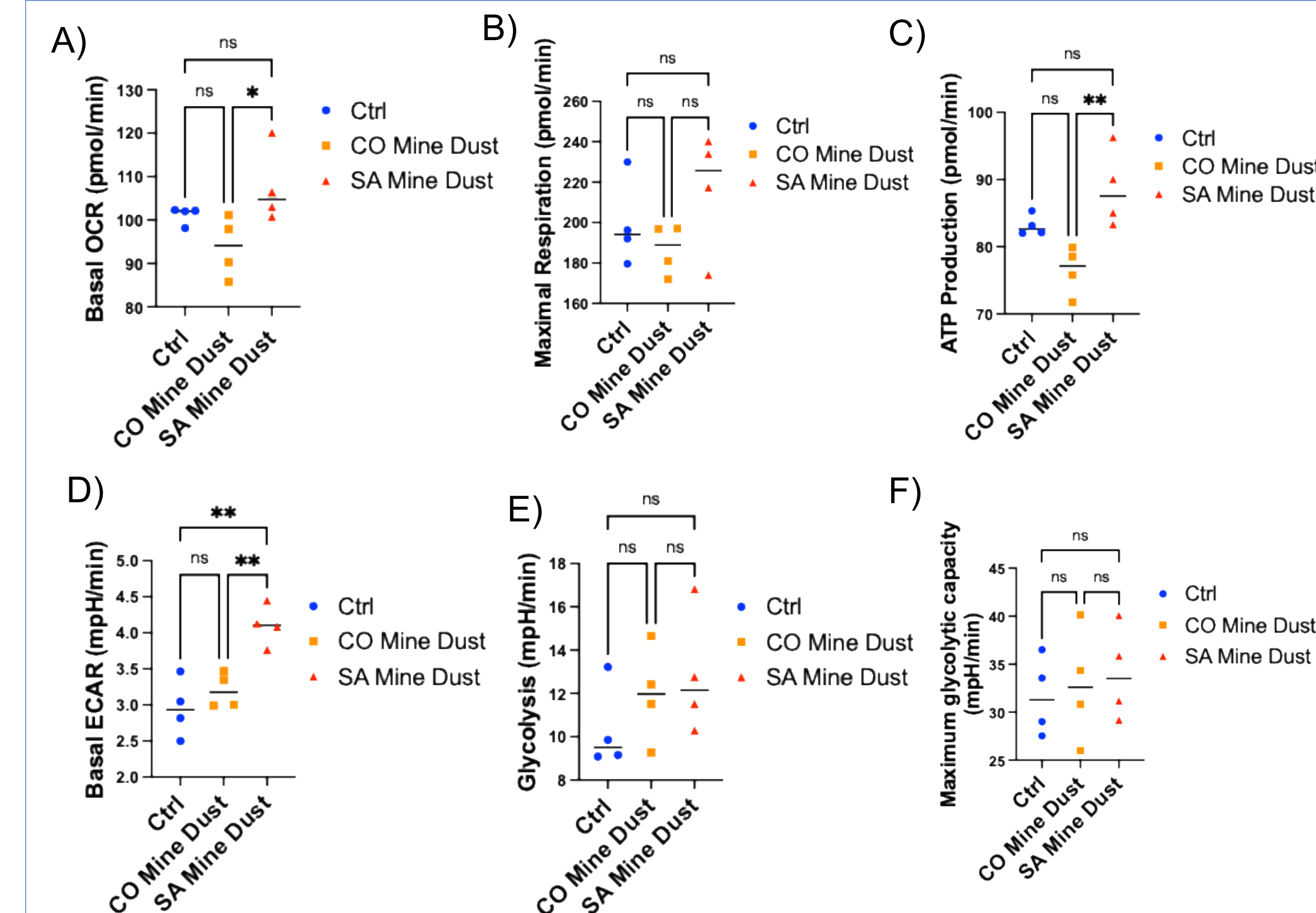


Figure 5. Bioenergetics in macrophages treated with control and mine-dust. St. Anthony mine dust-treated macrophages demonstrated a more statistically significant metabolic dysfunction than Colorado mine dust or vehicle-treated cells. This was prevalent in both the mitochondrial stress test (A-C) and glycolysis tests (D-F).

Conclusions

- Plasma EV-derived protein profiles were associated with U-mining tenure independent of age (Table 1 and 2)
- U-dust (St. Anthony mine) treated macrophages resulted in the greatest cellular dysfunction based on phagocytosis, mitochondrial stress and glycolysis stress tests.
- Future studies will examine the impact of extracellular vesicles on macrophage function.
- Further research is warranted to examine the molecular mechanisms of these findings.

This study is funded by NIEHS P42 ES025589 and R01 ES033981 with support from the UNM College of Pharmacy and the UNM Comprehensive Cancer Center. The authors would like to thank Dr. Pavan Muttill for usage of the cascade impactor for the dust fractionation.

Development of an Autoimmune Prone Disease State Following Inhalation Exposure to Uranium Bearing Dust in MRL/MpJ mice.

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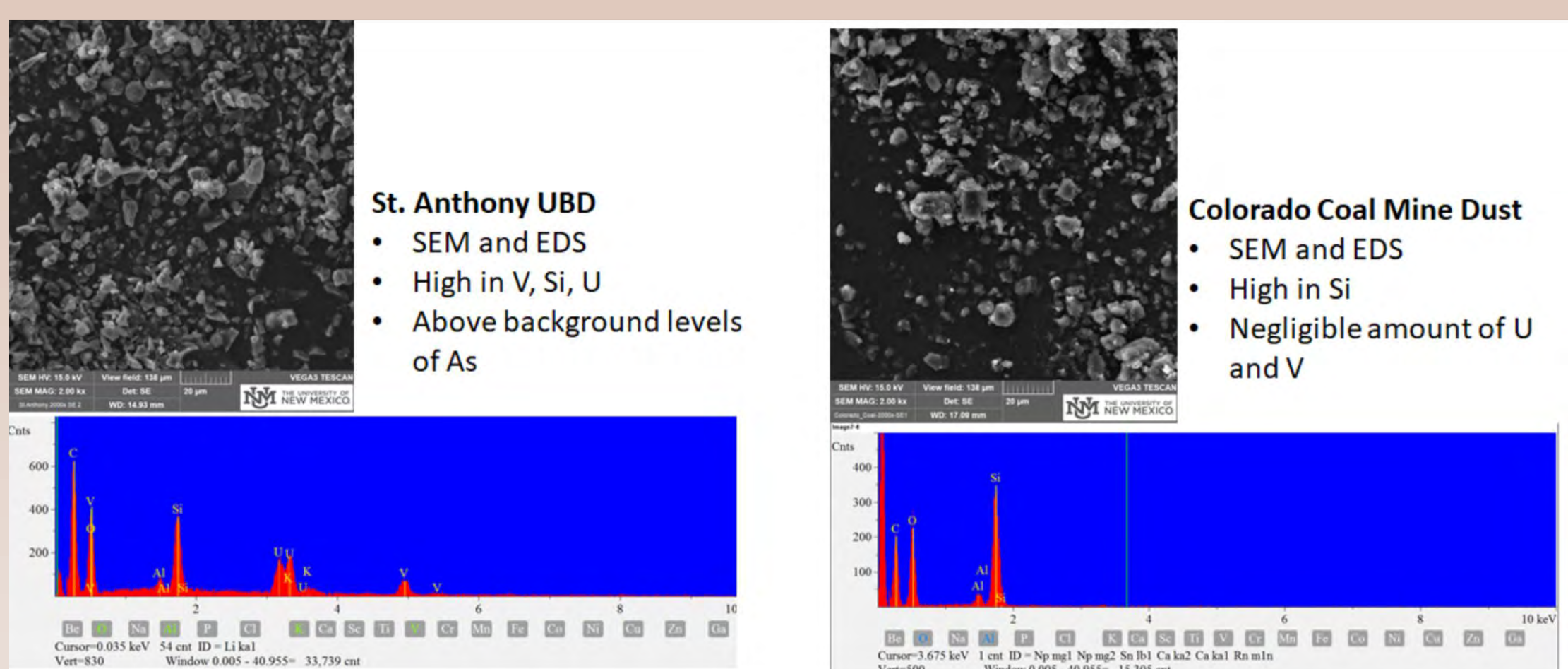
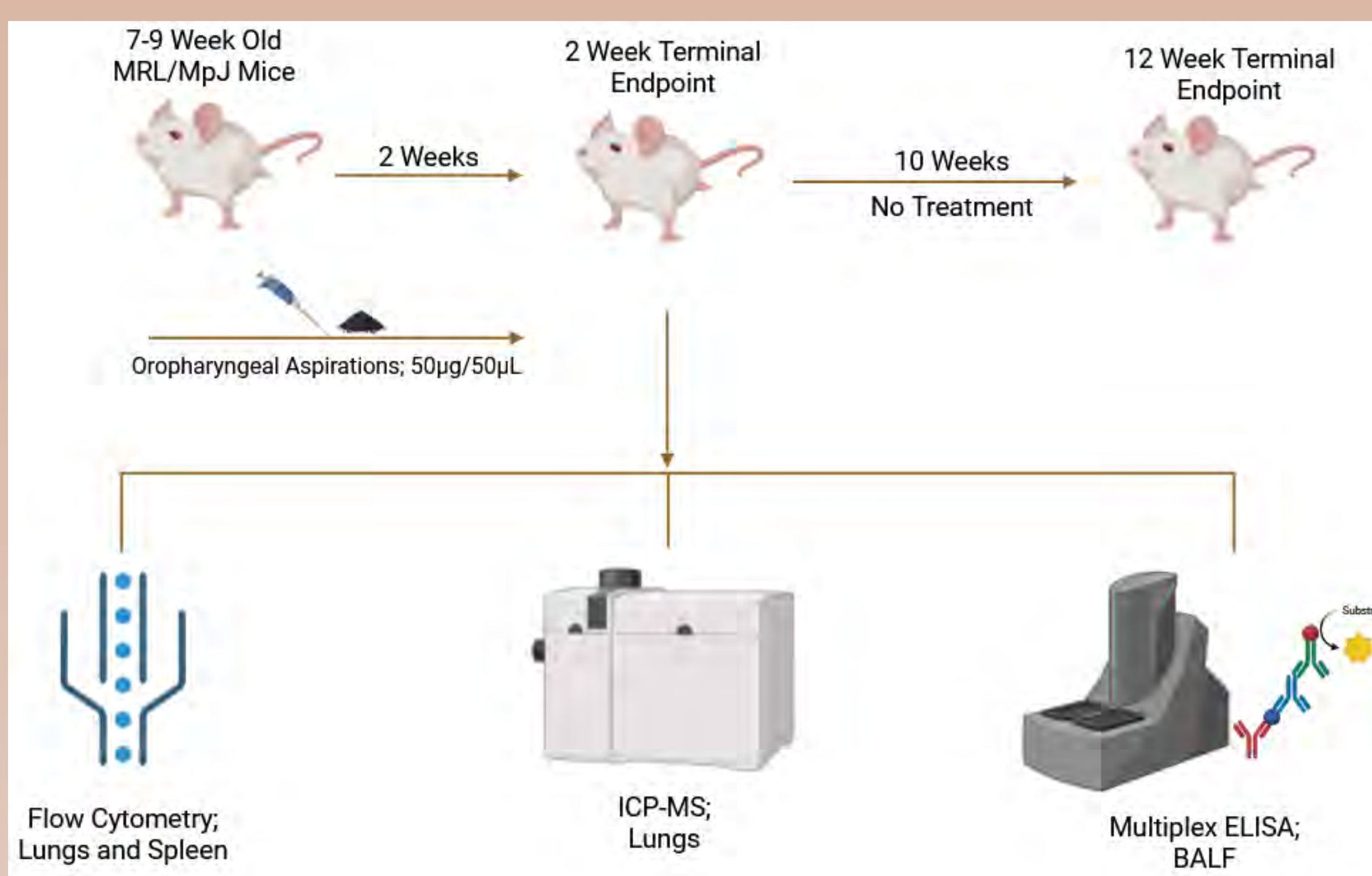
Introduction

- The western United States consists of thousands of abandoned uranium mines (AUMs).
- The Four Corners Region (Arizona, Utah, Colorado and New Mexico) contains many of these AUMs.
- Geographical proximity to the AUMs has a significant correlation with incidence of interstitial lung diseases, chronic obstructive pulmonary disorder, and autoimmune diseases, such as lupus and rheumatoid arthritis.
- How does inhalation exposure to AUM particulate matter affect the lungs and systemic immune responses?

Objective

- Utilizing the MRL/MpJ autoimmune susceptible mouse model, we aim to identify mechanisms of immune dysregulation and pathways of chronic inflammation that could lead to the development of autoimmune disorders following AUM particulate matter exposure.

Model and Exposure Paradigm



- 60 Female MRL/MpJ Mice
- 30 Mice for 2 Week Terminal Endpoint
- 30 Mice for 12 Week Terminal Endpoint
- 3 Exposure Groups
 - Dispersion Media Vehicle Control; DM
 - St. Anthony Uranium Bearing Dust (UBD)
 - Colorado Red Coal Mine Control Dust
- Immune Cell Population Profiling in Lungs (2 Week) and Spleens (12 Week) through Flow Cytometry
- Enzyme-linked Immunosorbent Assay profiling of cytokines in Bronchoalveolar Lavage Fluid (2/12 Week)
- Metal Content Quantification through Inductively Coupled Plasma-Mass Spectrometry on Lungs (2/12 Week)

Grants Mining District in New Mexico

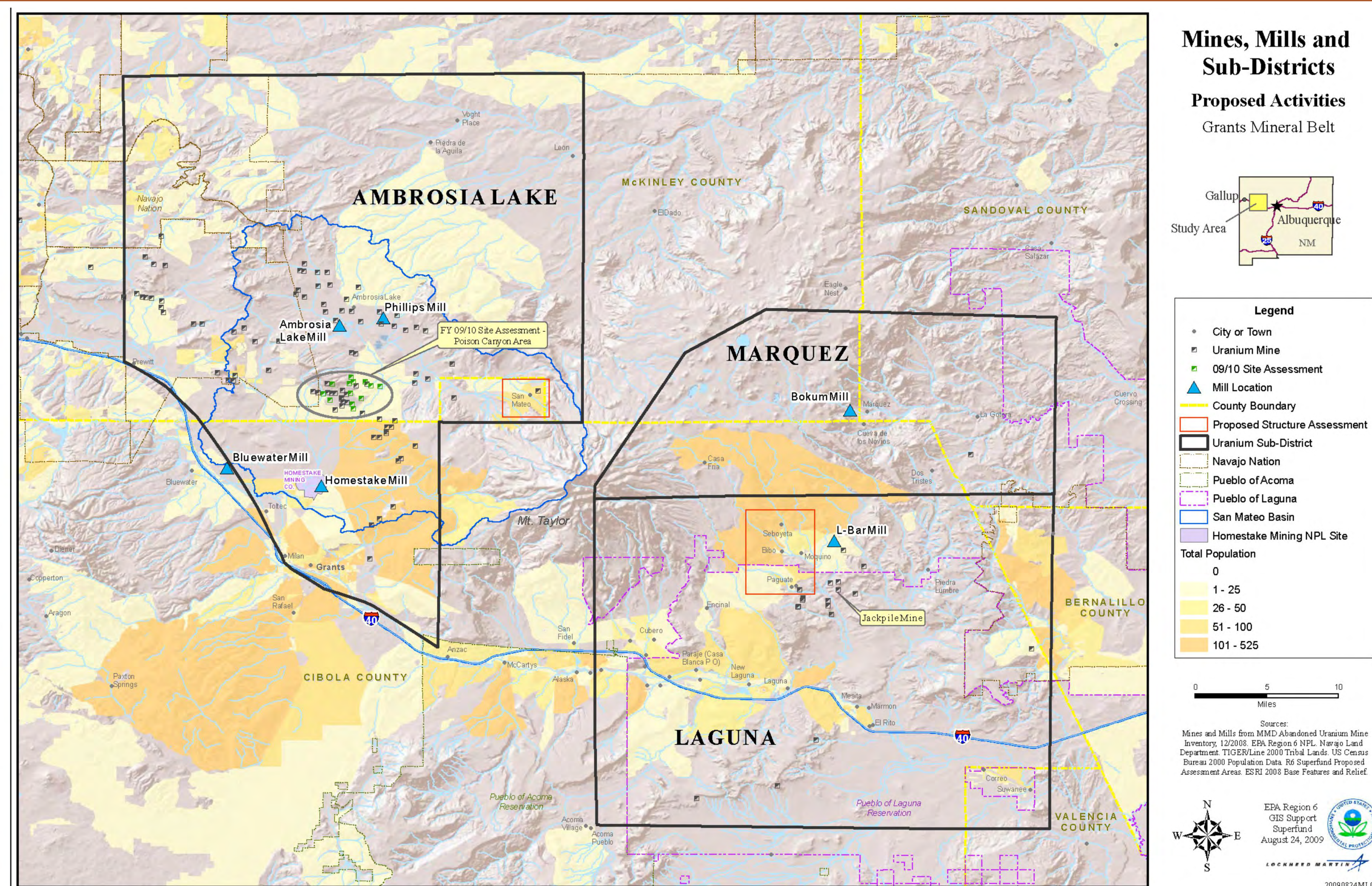


Figure 1: Map illustrating the Grants Mining District of New Mexico. St. Anthony Mine dust utilized in all experiments was gathered from the Laguna subdistrict in proximity to the Jackpile Mine indicated on the map. (Source: epa.gov)

Metals are Retained Within the Lung Weeks after Exposure to UBD

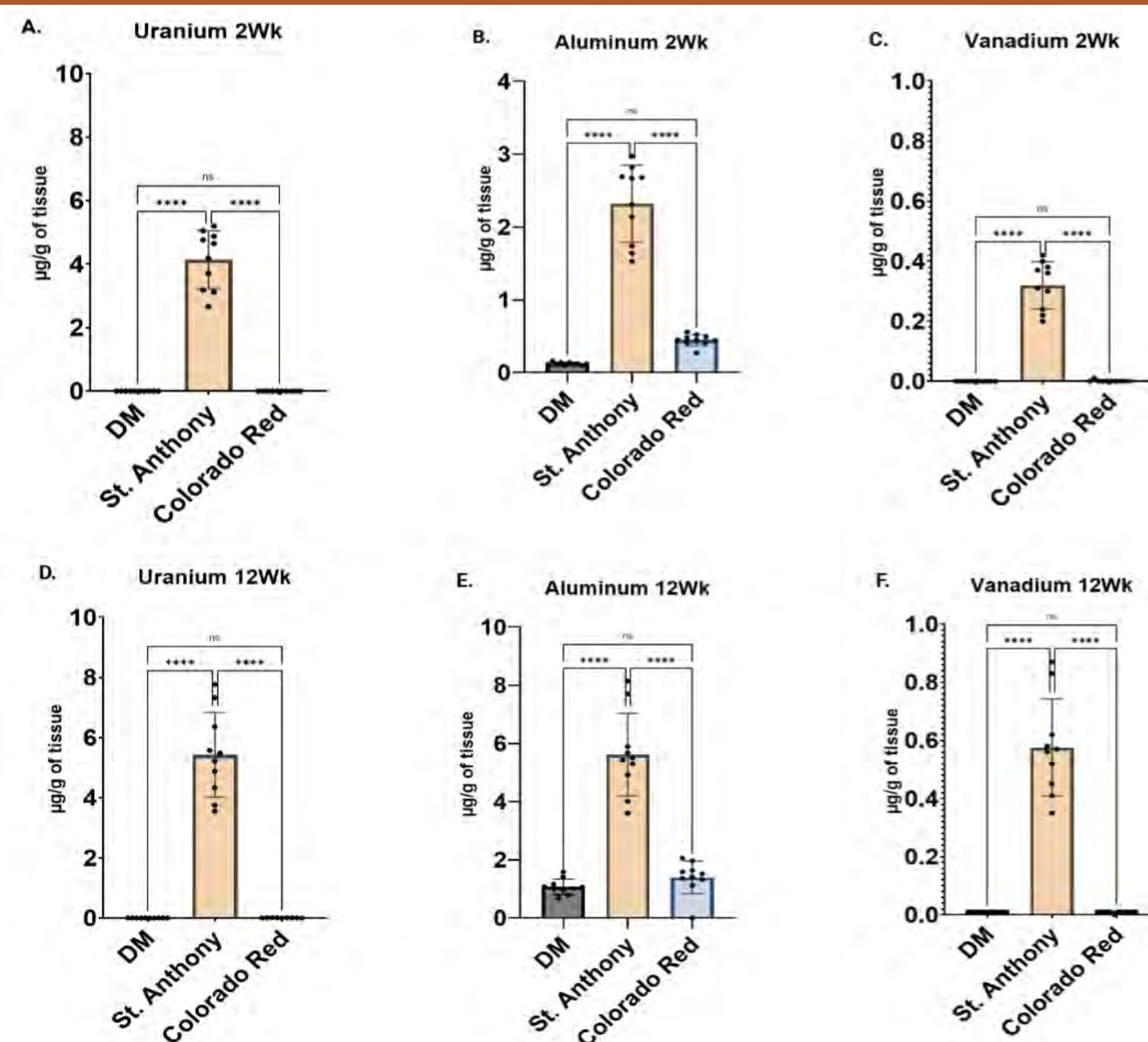


Figure 2: Retention of metals unique to the St. Anthony Mine Dust are retained up to 10 weeks after the cessation of exposure at 2 weeks. Uranium (A/D), Aluminum (B/E) and Vanadium (C/F), levels are maintained with no noticeable reduction in metal content between the time points. Analysis through one-way ANOVA, **** = $p < 0.0001$. DM = Dispersion Media Vehicle Control. St. Anthony = Uranium Bearing Dust. Colorado Red = Environmental Metal Control

Increased Cytokine and Chemokine Secretion in UBD Exposed Mice BALF

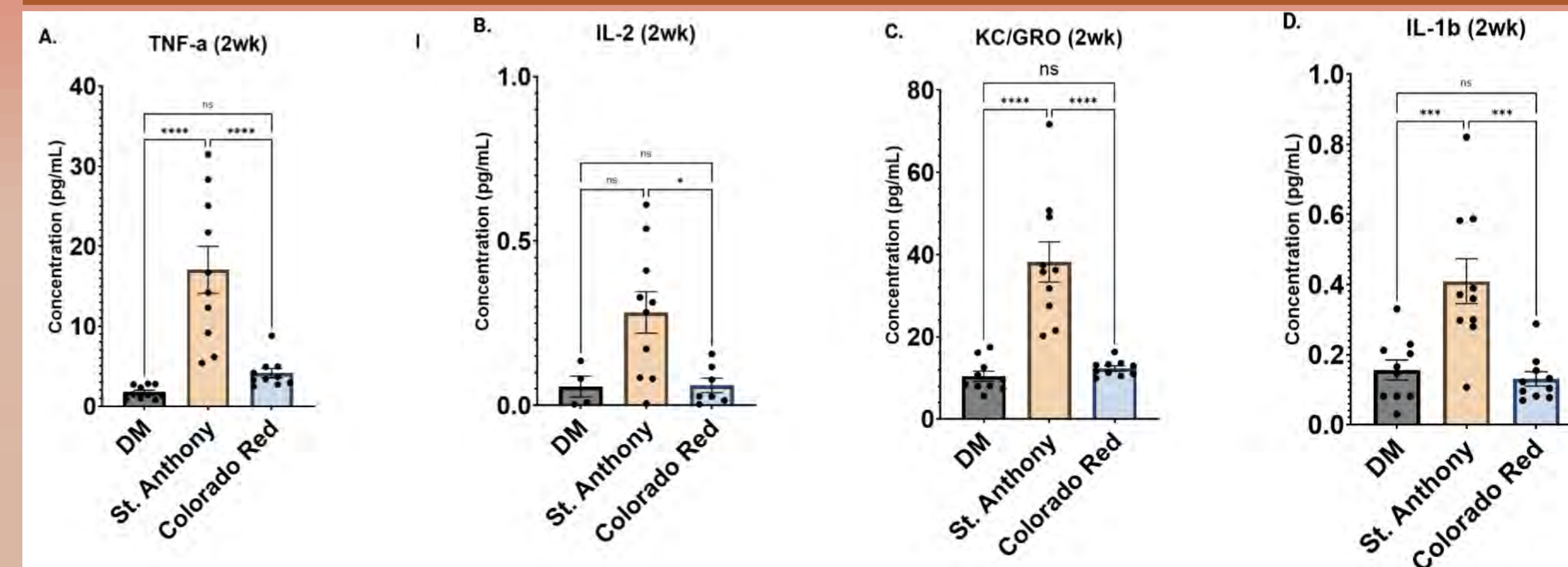


Figure 3: ELISA analysis of bronchoalveolar lavage fluid collected from the lungs of mice reveals a significantly increased secretion of cytokines that is unique to uranium bearing dust exposure. Cytokines related to pro-inflammatory pathway activations (A-B, D) as well as chemokine recruitment of neutrophils (C). Analysis through one-way ANOVA, *** = $p < 0.001$, **** = $p < 0.0001$. DM = Dispersion Media Vehicle Control. St. Anthony = Uranium Bearing Dust. Colorado Red = Environmental Metal Control

Increased Cytokine and Chemokine Secretion in UBD Exposed Mice BALF

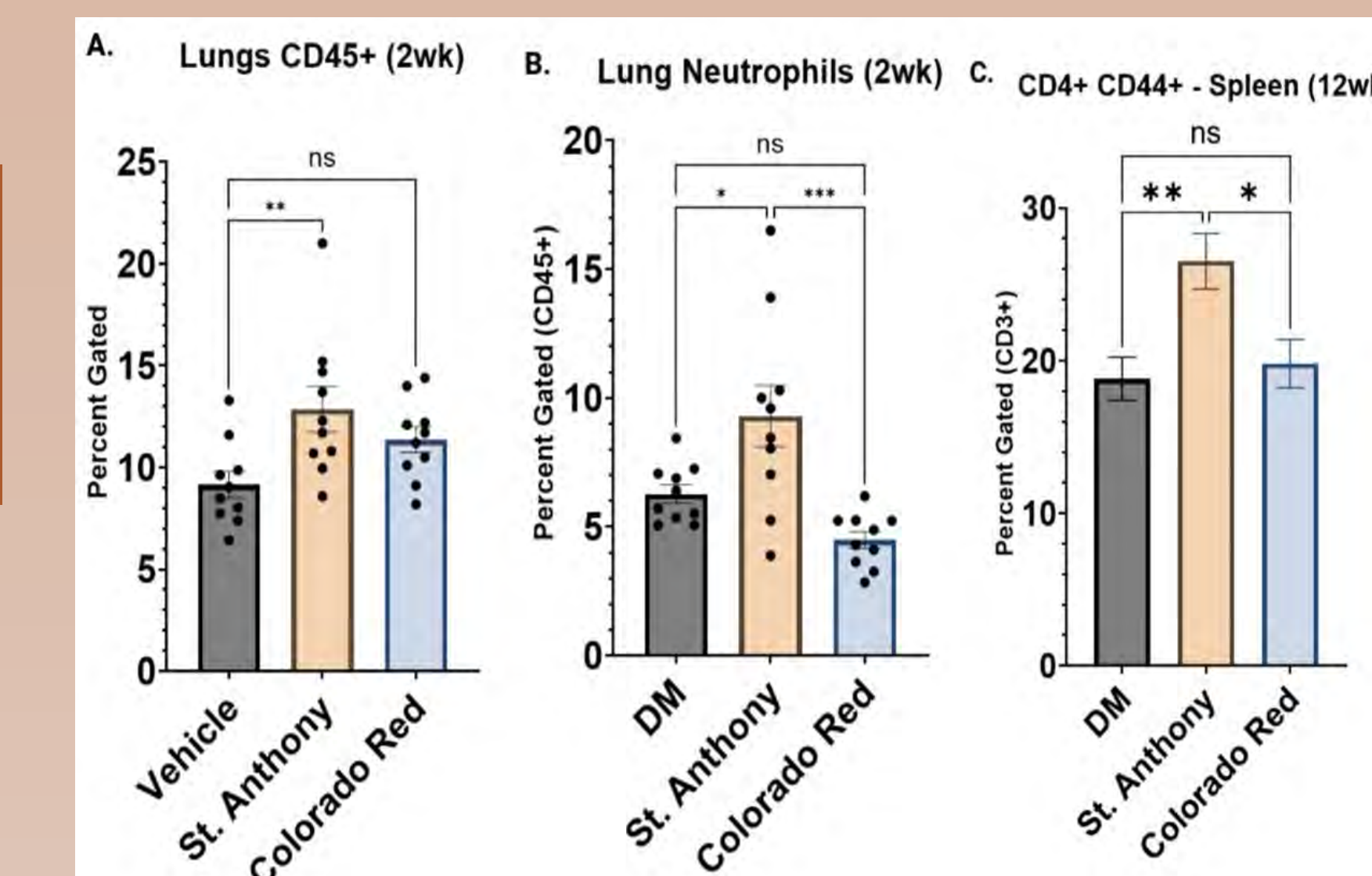


Figure 4: Exposure to St. Anthony AUM dust results in a local dysregulation of immune cell populations in the lung via recruitment of T Cells and neutrophils to the lung (A/B), and systemic alterations to T Helper Cells originating in the Spleen (C). Recruited neutrophils to the lung are active as is evident through their release of neutrophil elastase (E). * = $p < 0.05$, ** = $p < 0.005$, *** = $p < 0.001$. DM = Dispersion Media Vehicle Control. St. Anthony = Uranium Bearing Dust. Colorado Red = Environmental Metal Control

Neutrophils Exhibit Increased Activity After Exposure to UBD

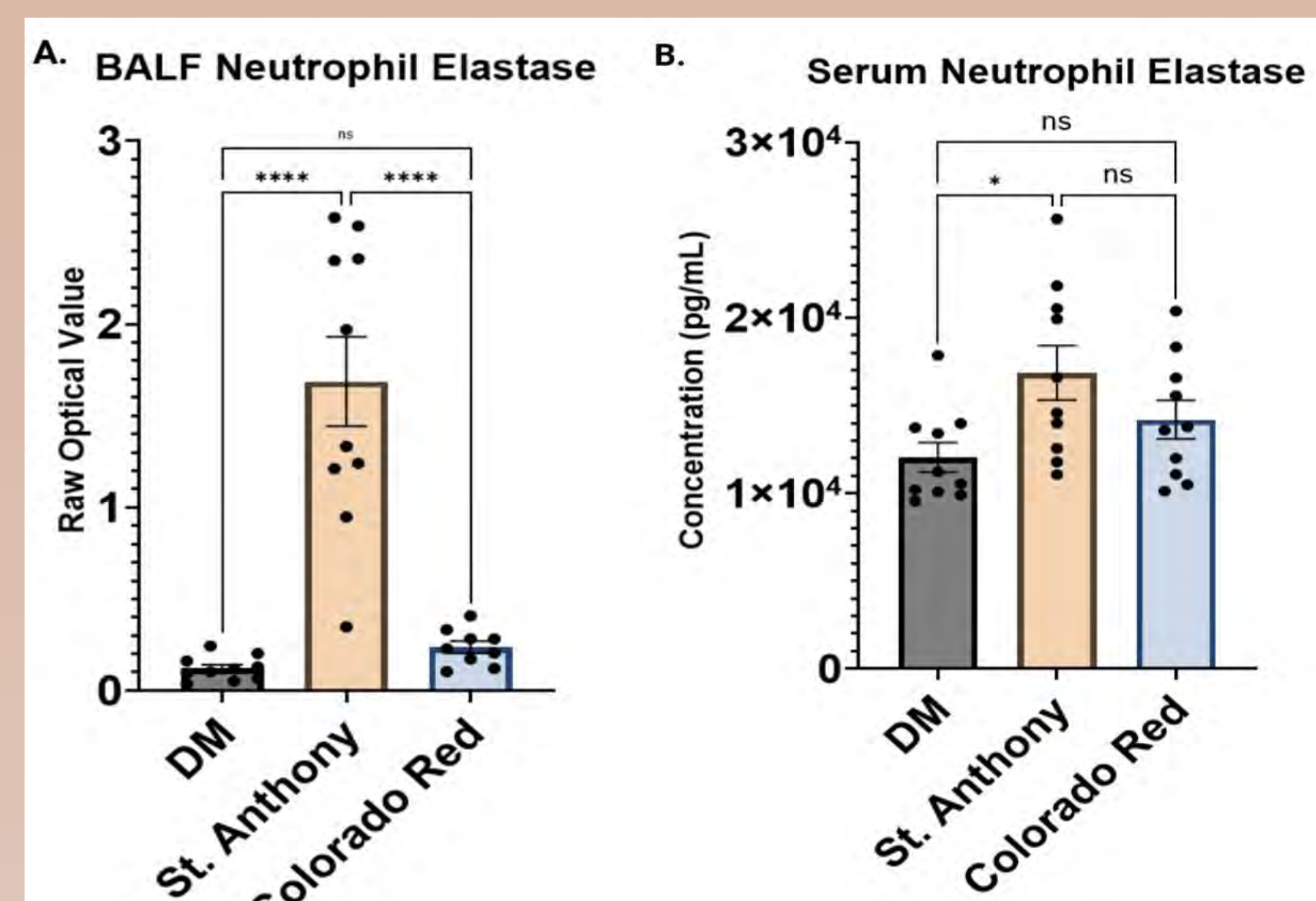
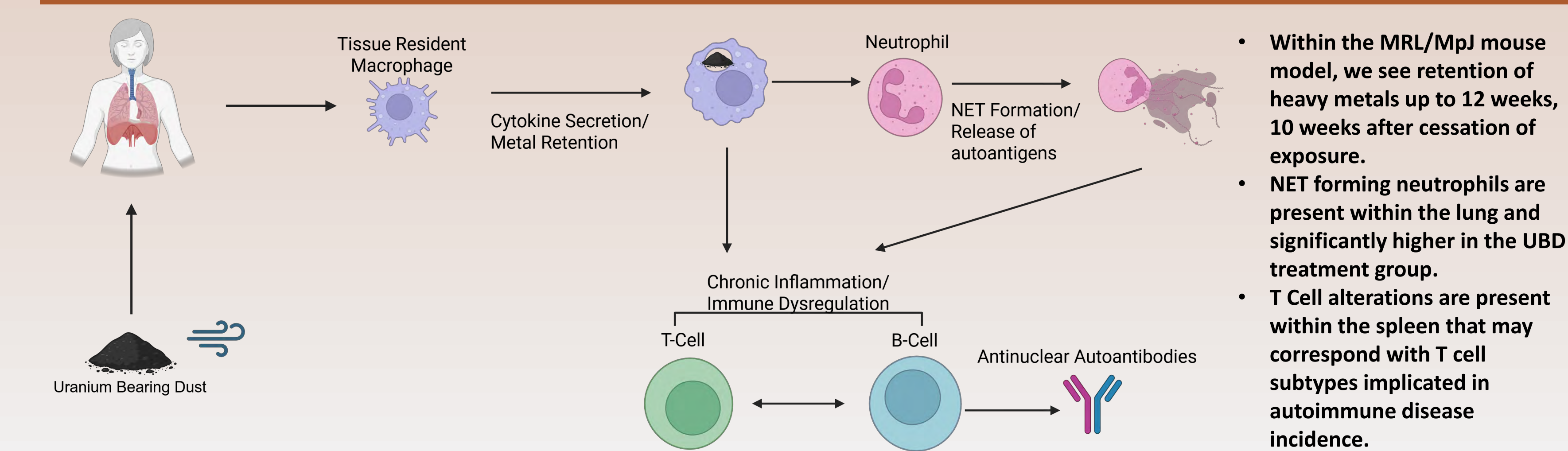


Figure 5: Neutrophils within the lung as well as the serum actively secrete neutrophil elastase, a key enzyme in the process of neutrophil extracellular trap release (A/B). * = $p < 0.05$, **** = $p < 0.0001$. DM = Dispersion Media Vehicle Control. St. Anthony = Uranium Bearing Dust. Colorado Red = Environmental Metal Control

Conclusions – Exposure Hypothesis

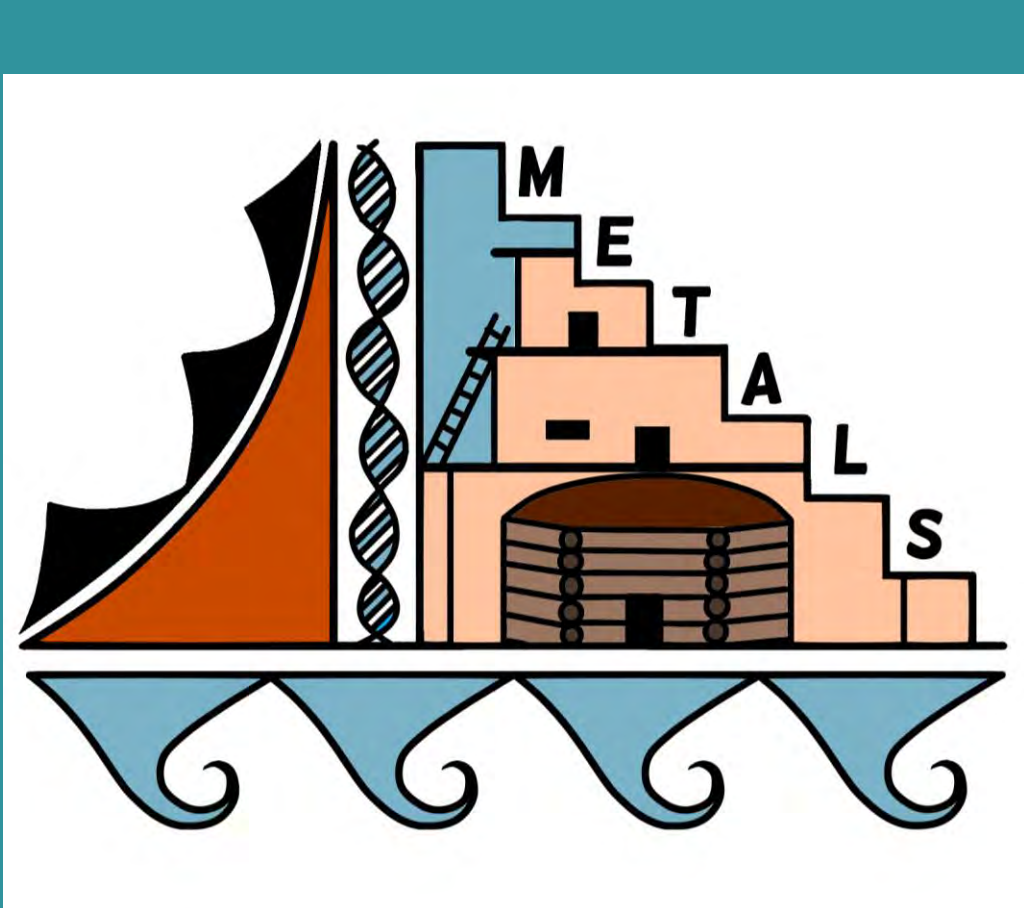


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ST32GM144834-02: Initiative to Maximize Student Diversity at the University of New Mexico Health Sciences Center 2021

Pueblo of Laguna Environmental and Natural Resources Department

This study is funded by NIEHS P42 ES025589 with support from the UNM College of Pharmacy and the UNM Comprehensive Cancer Center



UNM METALS Superfund Research Center

Thinking Zinc: An Intervention study for mitigation of metal toxicity

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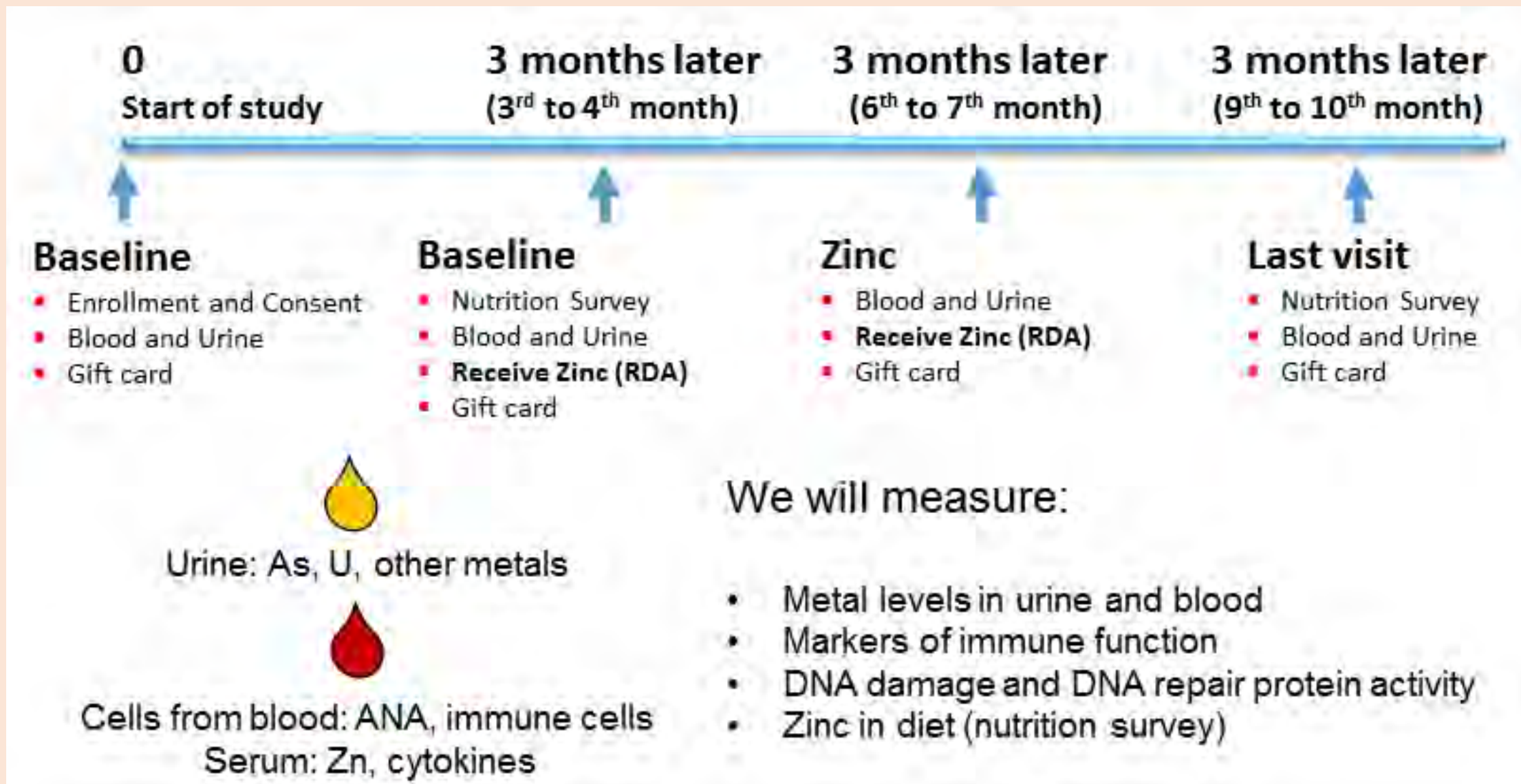


Abstract

Zinc is an essential micronutrient with various key functions in human health. Experimental models indicate that zinc protects against genotoxicity through multiple mechanisms including its functions in antioxidant defense and DNA damage repair pathways. Numerous environmental metals share underlying mechanisms of toxicity that contribute to adverse human health outcomes. Studies from our laboratory and others demonstrate that multiple metals disrupt zinc finger motifs altering protein function. In these systems, supplemental zinc confers protection against the metals' effects. Based on this evidence, a community and academic partnership developed an intervention trial called Thinking Zinc. Thinking Zinc is a mechanism-informed intervention study of zinc supplementation to test the potential benefits of zinc in a mixed metal exposed tribal population living in proximity to more than 500 abandoned uranium mines. The study is a single-arm cohort design with longitudinal collection of biospecimens. We investigated three biomarkers of DNA damage previously reported to be zinc responsive: DNA strand break (Comet assay), oxidative DNA damage (8-hydroxy-2-deoxyguanosine, 8-OHdG) and poly(ADP-ribose) polymerase (PARP) activity. Urinary metal analysis finds Thinking Zinc study participants with elevated levels of multiple environmental metals compared to the general US population, including some that had at least 10% of the participants above the National Health and Nutrition Examination Survey (NHANES) 95th percentile. Metals concentrations showed substantial fluctuations over time, with greater differences detected in urinary metals than in serum metals. Zinc supplementation did not appreciably impact urinary levels of measured metals nor significantly increase serum zinc in study participants. However, zinc supplementation did modulate DNA damage markers evaluated by comet assay and PARP activity. We conclude that zinc is an important determinant of DNA damage in human populations and that DNA damage biomarkers are not equivalent for detecting the impact of zinc on DNA damage.

Overall Question and Study Design

Question: Will findings of zinc benefit from experimental models be applicable to a human population with environmental metal exposures?

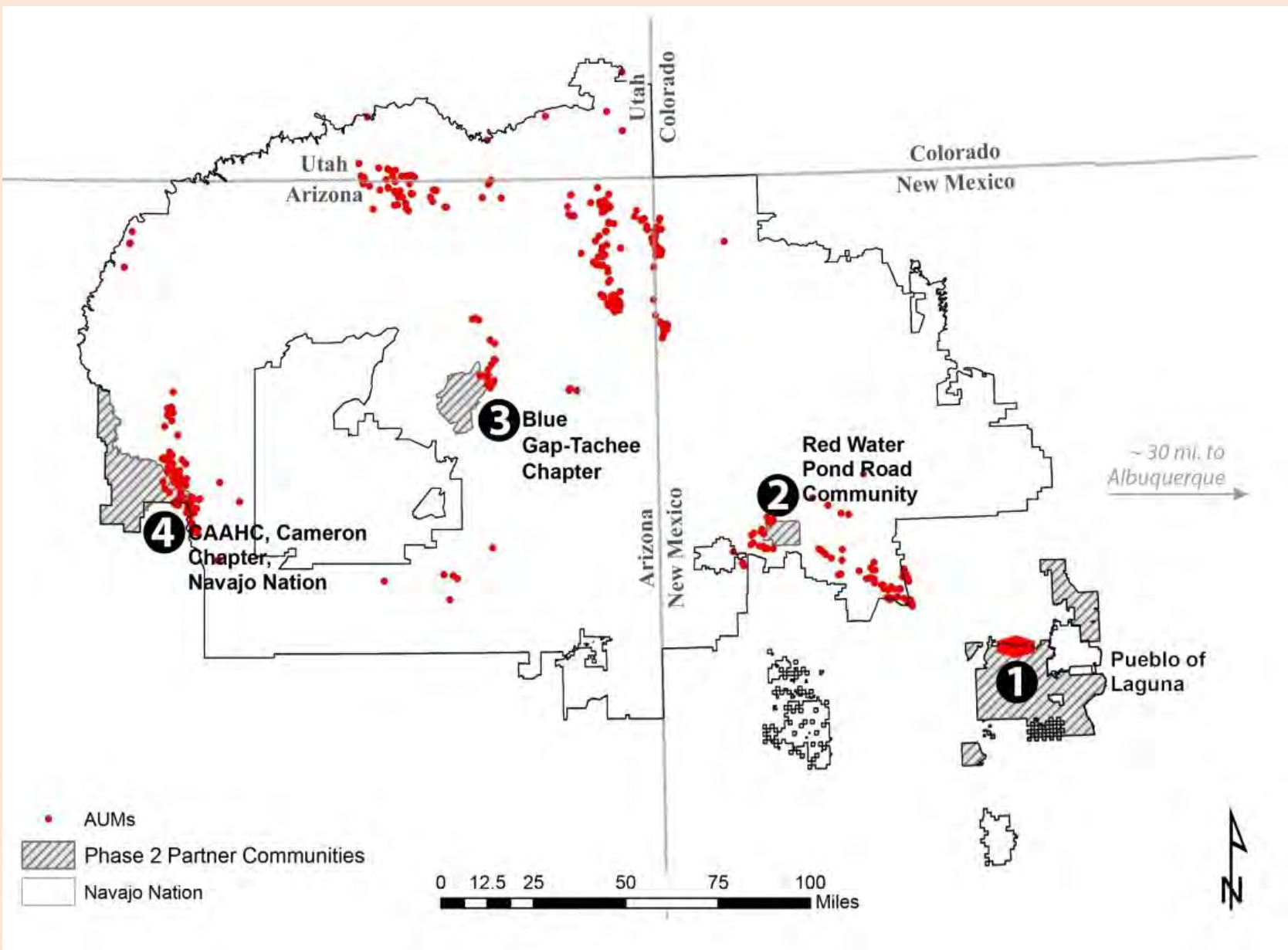


Inclusion criteria:

- Men or women between the ages of 21-64
- Current resident of the Navajo Nation.
- Willing to provide blood and urine samples on scheduled study dates.
- Willing to take a daily zinc supplement.

Exclusion criteria:

- Women who are pregnant or nursing or women who plan to become pregnant.
- Diagnosed diabetes.
- Known or suspected allergy to zinc.
- Diagnosed syndrome of copper homeostasis.
- Individuals consuming zinc supplements or multivitamins and are unwilling to stop for the duration of the study.



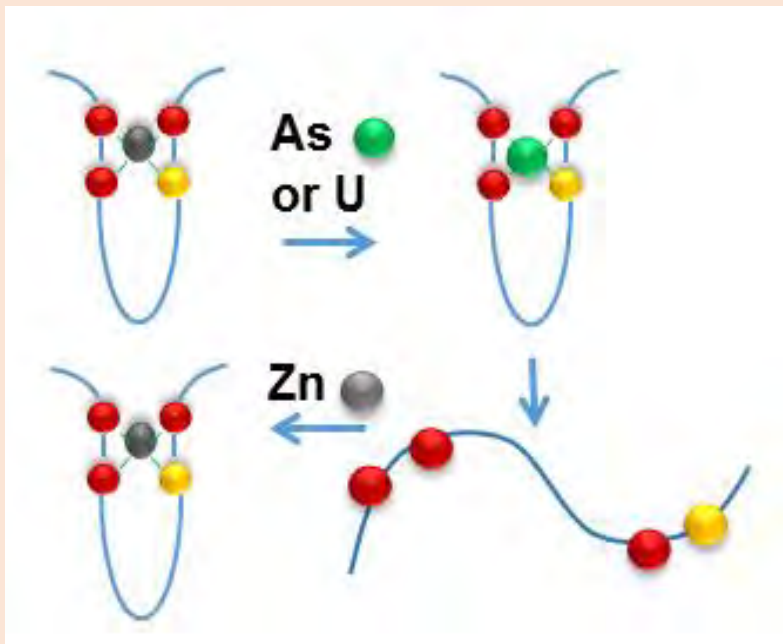
Why Zinc?

Zinc-binding proteins regulate many aspects of cell function.

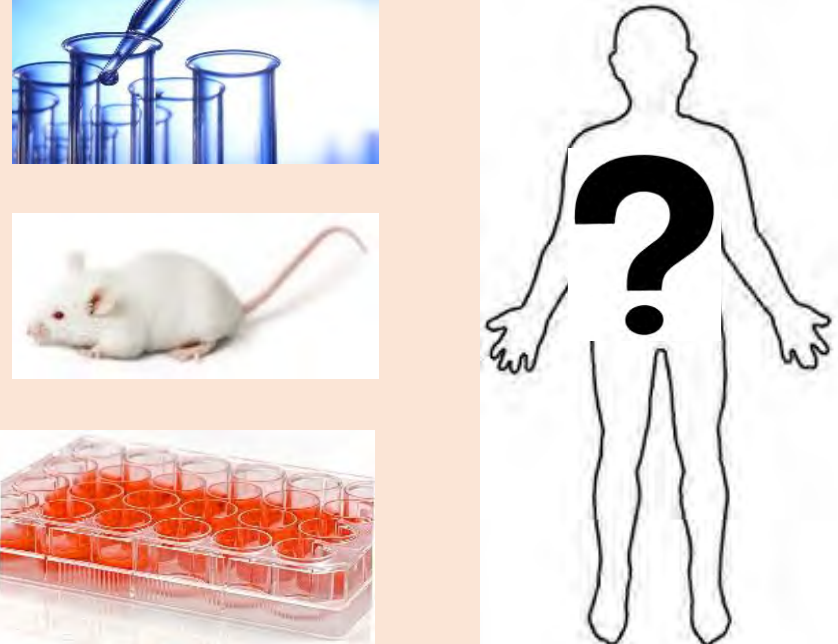


"Zinc Finger" Mallery Quetawki

Certain metals displace zinc in proteins*.



Zinc is protective in peptide, protein, cell and animal models*.



*>40 bench research publications by UNM METALS researchers.

- Low serum zinc is associated with increased DNA damage increased inflammatory cytokines in non-indigenous populations¹⁻⁴.
- Higher urinary arsenic is associated with increased oxidative stress which induces DNA damage⁶.

Preliminary Results

Study enrollment data

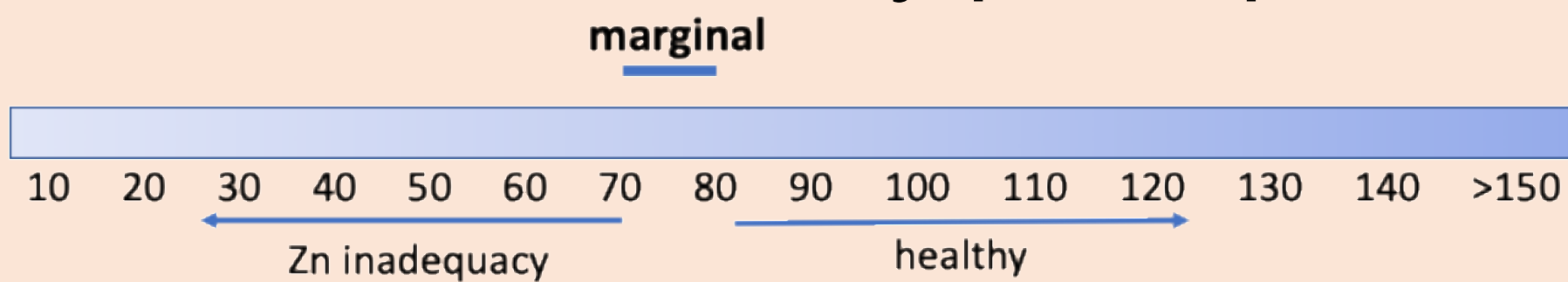
Current Enrollment	RWPR	Blue Gap-Tachee	Manuelito	Total
Enrolled M/F	37 (12/25)	15 (6/9)	19 (8/11)	71 (26M/45F)
Median Age	51 (21-64)	56 (23-63)	52 (32-64)	53.0 (21-64)

Study participants have elevated urinary metals

Table 1. Subset of Thinking Zinc Participant Pre-Zinc Urinary Metal Levels					
Metal	Median	Range	%>95 th percentile NHANES	NHANES 50 th	NHANES 95 th
Antimony	0.058	0.004 – 1.540	9.6%	0.046	0.151
Arsenic	4.305	0.893 – 135.014	2.1%	5.62	56.2
Barium	0.981	0.025 – 437.268	4.25%	1.24	4.83
Cadmium	0.135	0.009 – 1.207	3.2%	0.188	0.882
Cesium	2.937	0.055 – 25.151	4.3%	4.22	10.4
Cobalt	0.288	0.019 – 4.794	5.3%	0.404	1.2
Lead	0.153	0.002 – 19.164	6.4%	0.315	1.14
Manganese	0.096	0.002 – 2.878	9.6%	0.209	0.487
Molybdenum	17.832	0.363 – 130.684	1.1%	36.3	94.7
Platinum	0.0145	0.000 – 0.398	21.3%	<LOD	0.035
Strontium	74.794	0.961 – 3101.055	7.5%	101	266
Tin	0.711	0.010 – 14.606	9.6%	0.431	3.06
Uranium	0.021	0.002 – 19.164	41.5%	0.005	0.026
Vanadium*	0.159	0.019 – 28.837			

Median metal levels are shown for Visit 1 and Visit 2 samples (n = 94) collected before zinc supplementation. Values are corrected for urinary creatinine and reported as micrograms per gram creatinine (µg/g creatinine). For reference, the 50th and 95th percentile levels are provided for the 2019 (January) National Health and Nutrition Examination Survey (NHANES) values for each metal. Metals results highlighted in blue represent those where more than 10% of samples had levels in excess of the NHANES 95th percentile values. *Urine levels for vanadium are not included in NHANES reporting. "<LOD" – Not available due to measurements below the level of detection of the instrument

Serum zinc levels in study participant samples



≤ 80 µg/dL marginal sufficiency*

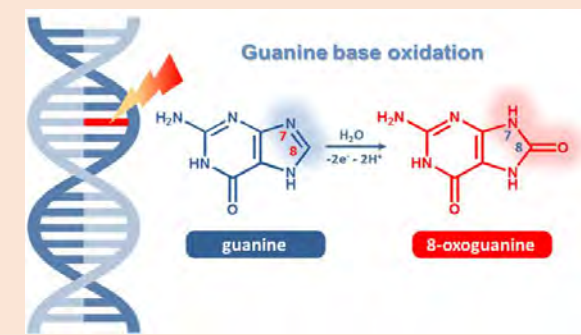
- More women than men
- Improvement with Zn supplement
 - 31.3% pre Zn
 - 13.7% post Zn

≤ 70 µg/dL Zn inadequate

- Modest improvement with Zn supplement
 - 11.9% pre Zn
 - 7.3% post Zn

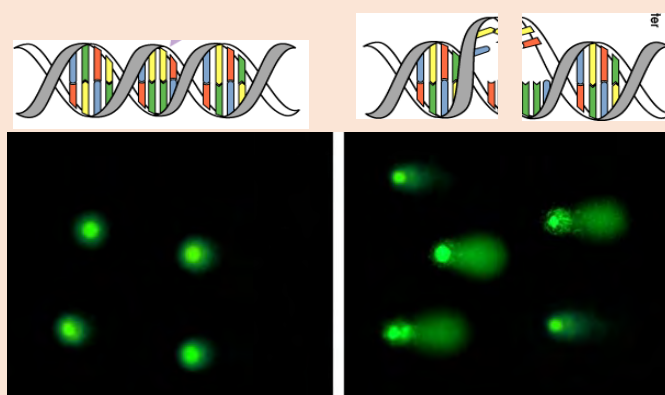
DNA Damage Biomarkers measured

Oxidative DNA Damage



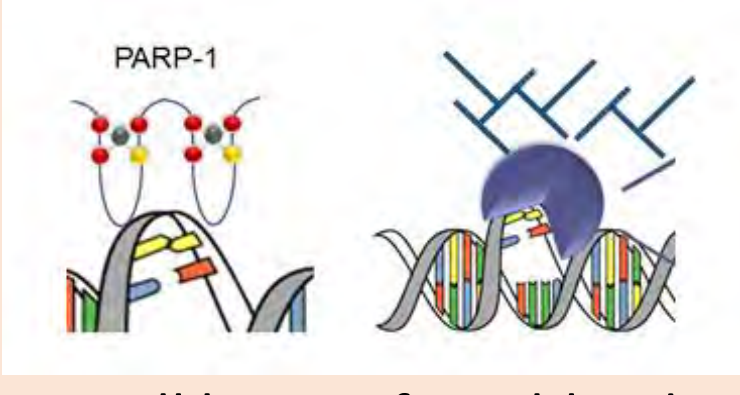
Urine/ELISA

DNA Strand Break



Cells from blood samples
Comet Assay

PARP Activity



Cell lysates from blood
samples
ELISA

Table 2. Participant DNA damage biomarkers pre- and post-zinc			
	Pre-Zinc (IQR)	Post-Zinc (IQR)	P value
Oxidative DNA Damage	19.2 (13.8, 26.1)	15.3 (10.5, 27.0)	0.3
(Typical reference range of 2.7-13 ng/mg creatinine, mean 8.4 ng/mg creatinine)			
DNA Strand Break (Tail moment mean)	0.37 (0.09, 0.89)	0.22 (0.13, 0.44)	0.004
(No reference ranges available)			
PARP Activity	388 (274, 434)	250 (190, 294)	<0.001
(No reference ranges available)			

Table 3. Sex differences observed in DNA damage biomarkers pre- and post-zinc

	Female (N=72) Pre/Post-Zinc	Male (N=45) Pre/Post-Zinc
Oxidative DNA Damage	P=0.3	P=0.8
DNA Strand Break (Tail moment mean)	P=0.2	P=0.002
PARP Activity	P<0.001	P<0.001

Table 3. Community differences observed in DNA damage biomarkers pre- and post-zinc

	Community	Pre/Post-Zinc	Trend direction post-zinc
Oxidative DNA Damage	RWPR	P=0.05	↓
	BG-Tachee	P=0.2	NC
	Manuelito	P=0.2	↓
DNA Strand Break (Tail moment mean)	RWPR	P=0.2	↓
	BG-Tachee	P=0.2	↓
	Manuelito	P<0.001	↓
PARP Activity	RWPR	P<0.001	↓
	BG-Tachee	P<0.001	↓
	Manuelito	P<0.001	↓

Conclusions

- Zinc supplementation did not impact oxidative DNA damage; however, zinc did reduce DNA strand break and PARP biomarkers when assessing the entire cohort. The effects were even more pronounced when analyzing the impact of zinc supplementation on PARP activity by sex.
- Changes in biomarker responses were evident even with modest (RDA) zinc supplementation.
- The results support further investigation of Zn supplementation in populations exposed to environmental metals.

Acknowledgements

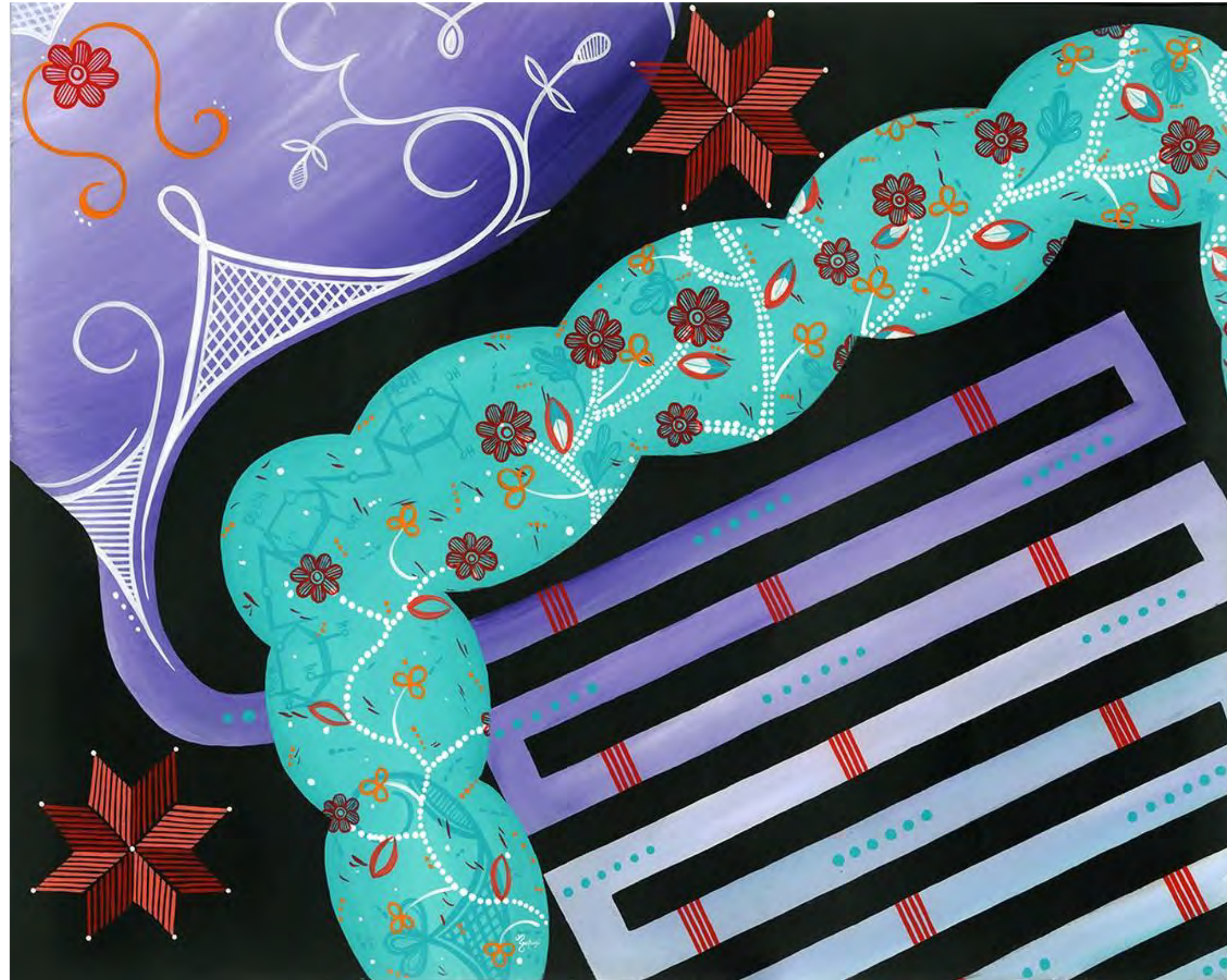
We thank the Navajo Nation and communities of Red Water Pond Road, Blue Gap-Tachee, and Manuelito for contributing to and supporting this work!

Approvals: The study Thinking Zinc is approved by the University of New Mexico Institutional Review Board (#18-381) in December 2018 and by the Navajo Nation Human Research Review Board (# NNR 18-330) in January 2019

Funding: NIEHS P42ES025589

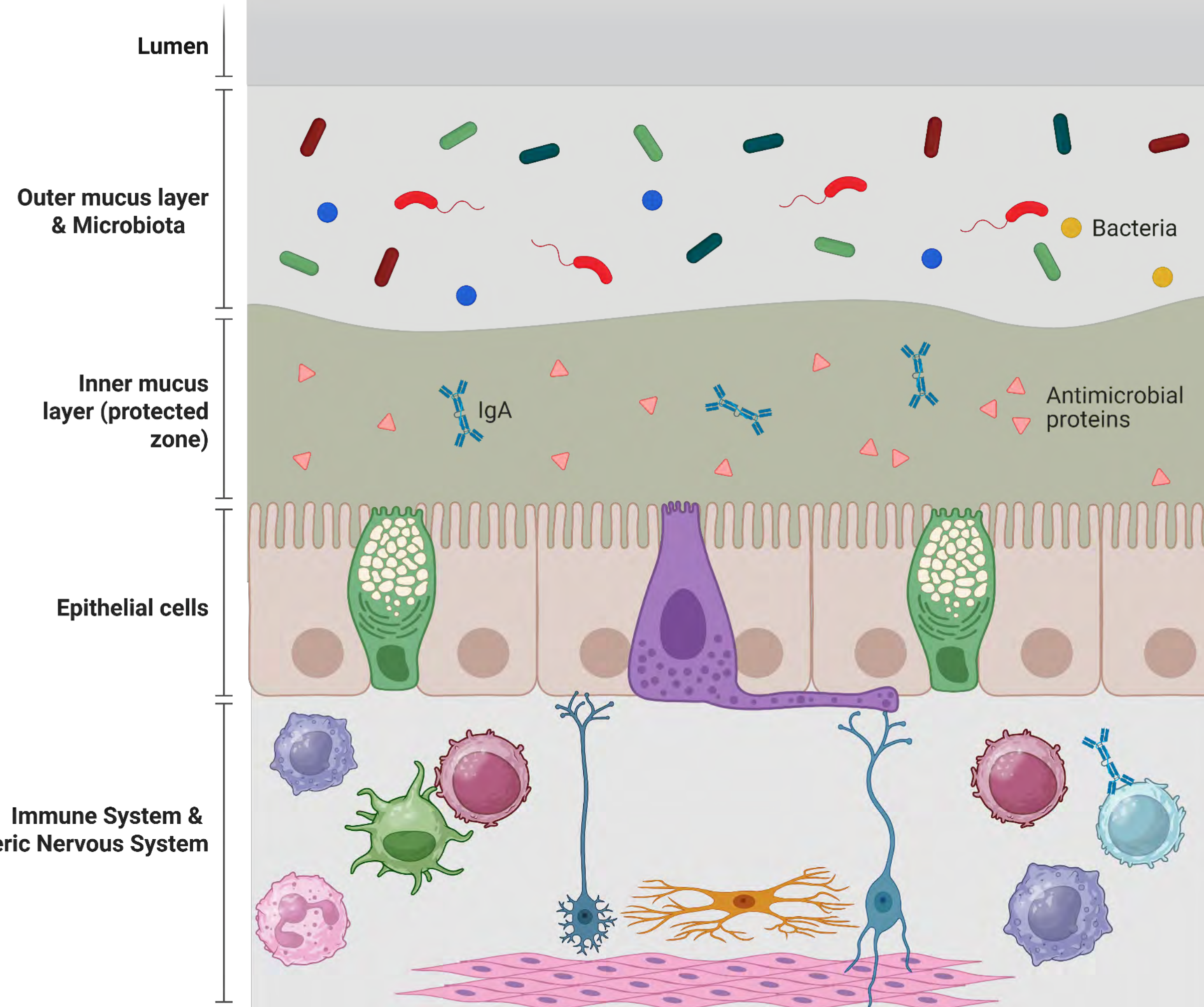
Additional Funding from: CDC U01 TS000135 (NBCS), NIEHS & NIMHD P50ES026102 (Native EH Equity), USEPA 83615701 (Native EH Equity Center), UNM Comprehensive Cancer Center NCI P30CA11810, UNM College of Pharmacy, New Mexico Technology Enhancement Fund FY24

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other funding agencies

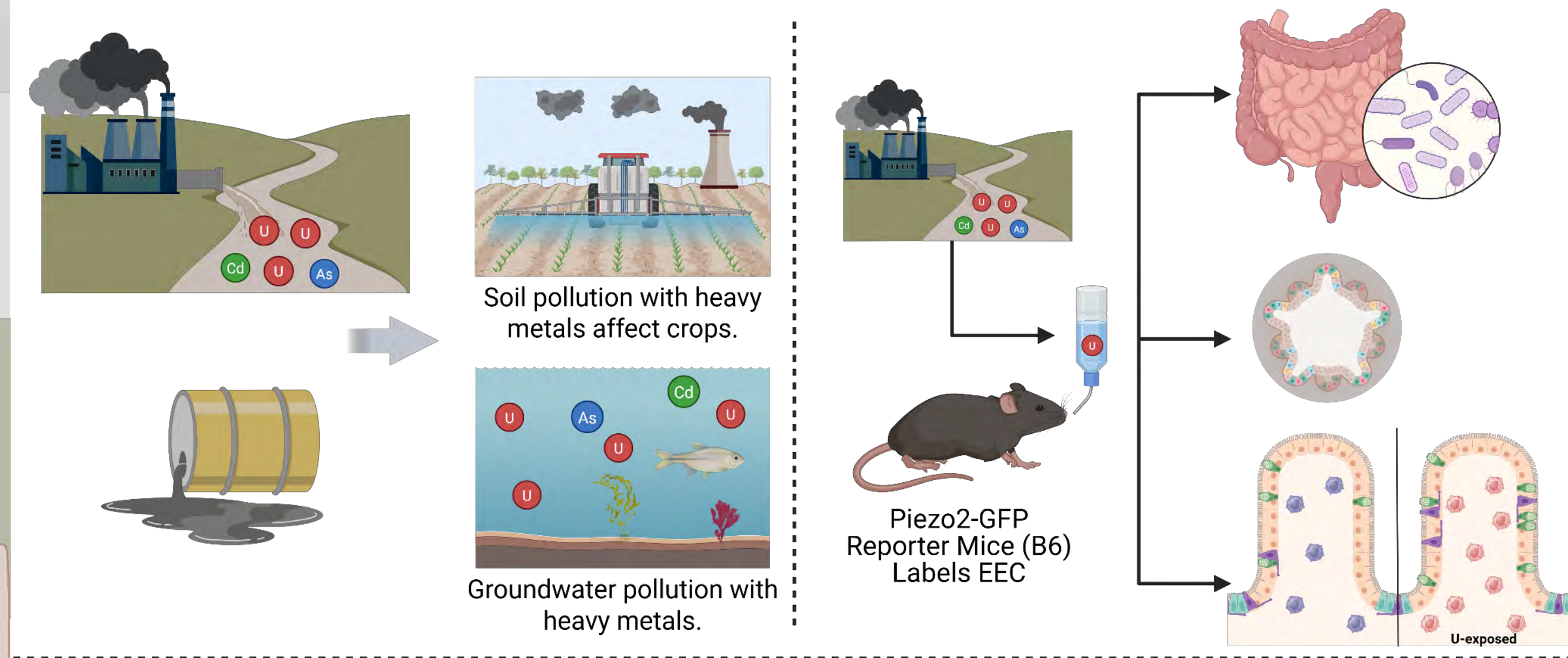


“Our Flora” 2020 24x30 in Acrylic on Canvas. Mallery Quetawki (Zuni Pueblo). Our gut microbiome is populated by diverse communities of bacteria that can change the environment of the digestive tract based on levels of these microbes and the role of prebiotics. To help explain the processes and components of gut flora, beadwork methods were used to help describe bacterial bacilli and rods. This painting in particular was created for the Dawn Land peoples of Northeast Coast, so the Wabanaki flower patterns are presented here as probiotic flora. The loose and incomplete design on the bottom left of the large intestine becomes whole as it passes through a chain of oligosaccharides (prebiotics) right above it.

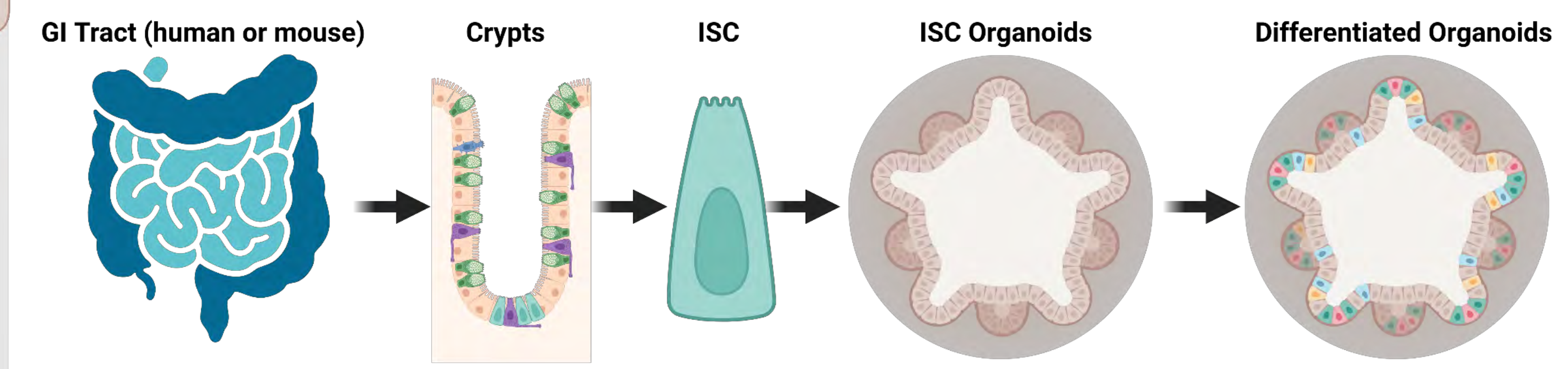
The Layers of the Gastrointestinal Tract: Microbiota, Epithelial Cells, Immune Cells and the Enteric Nervous System



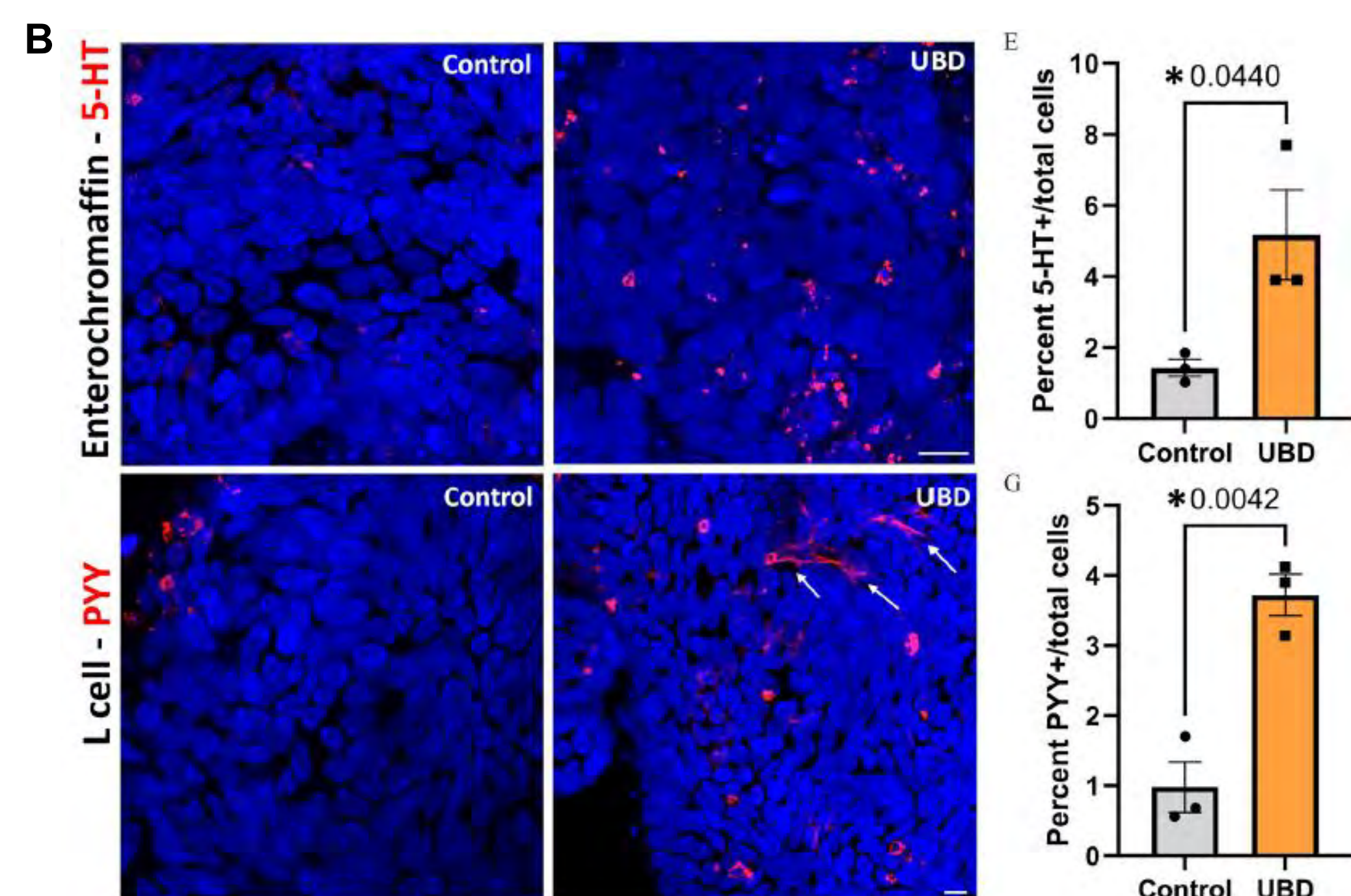
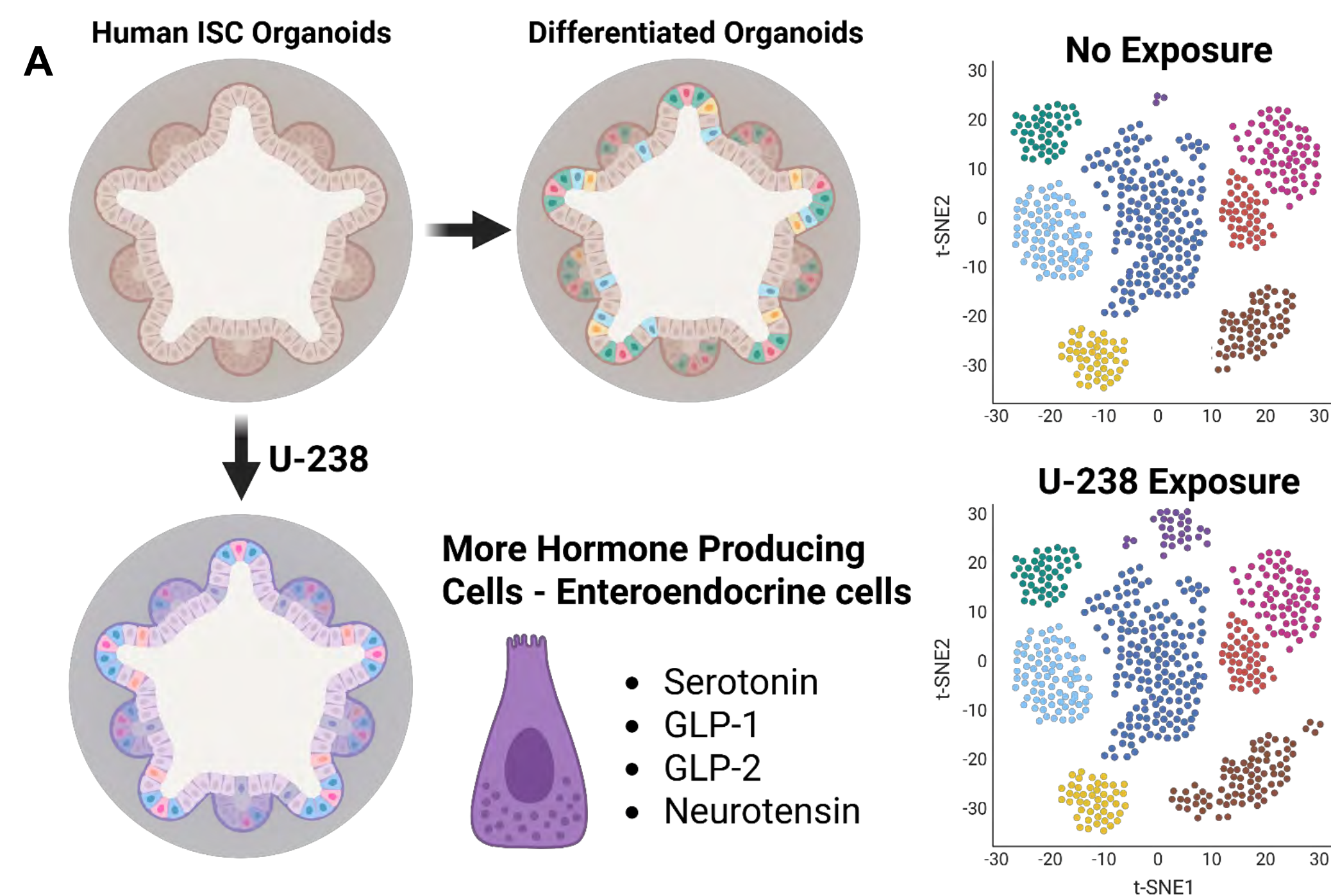
P42 METALS BP GUT Examining the effects of heavy metals on the Gastrointestinal Tract



GENERATION OF INTESTINAL ORGANOIDS

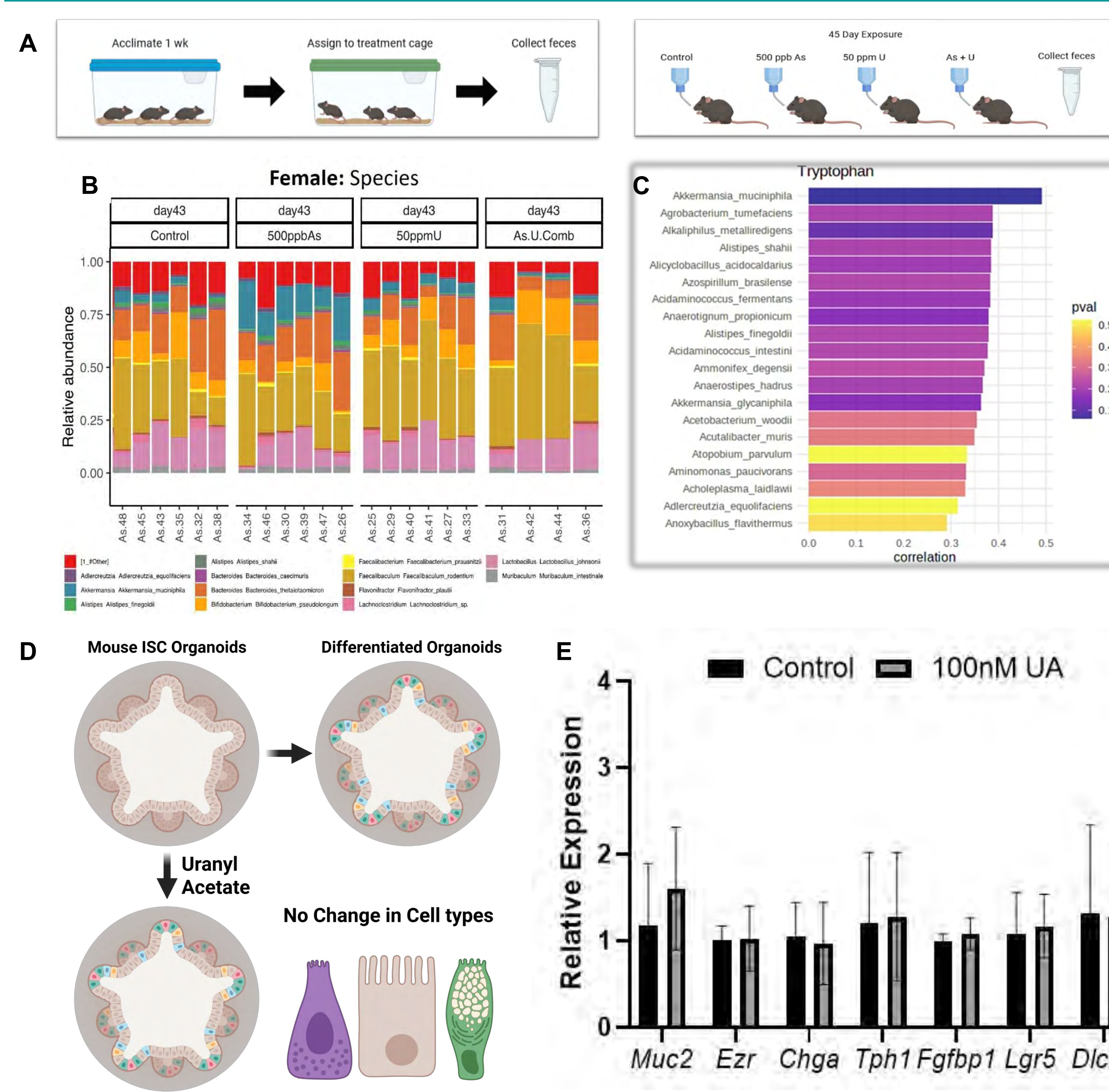


What is Uranium doing to the Gut Epithelium? Using Human Intestinal Organoids: Mini-Guts



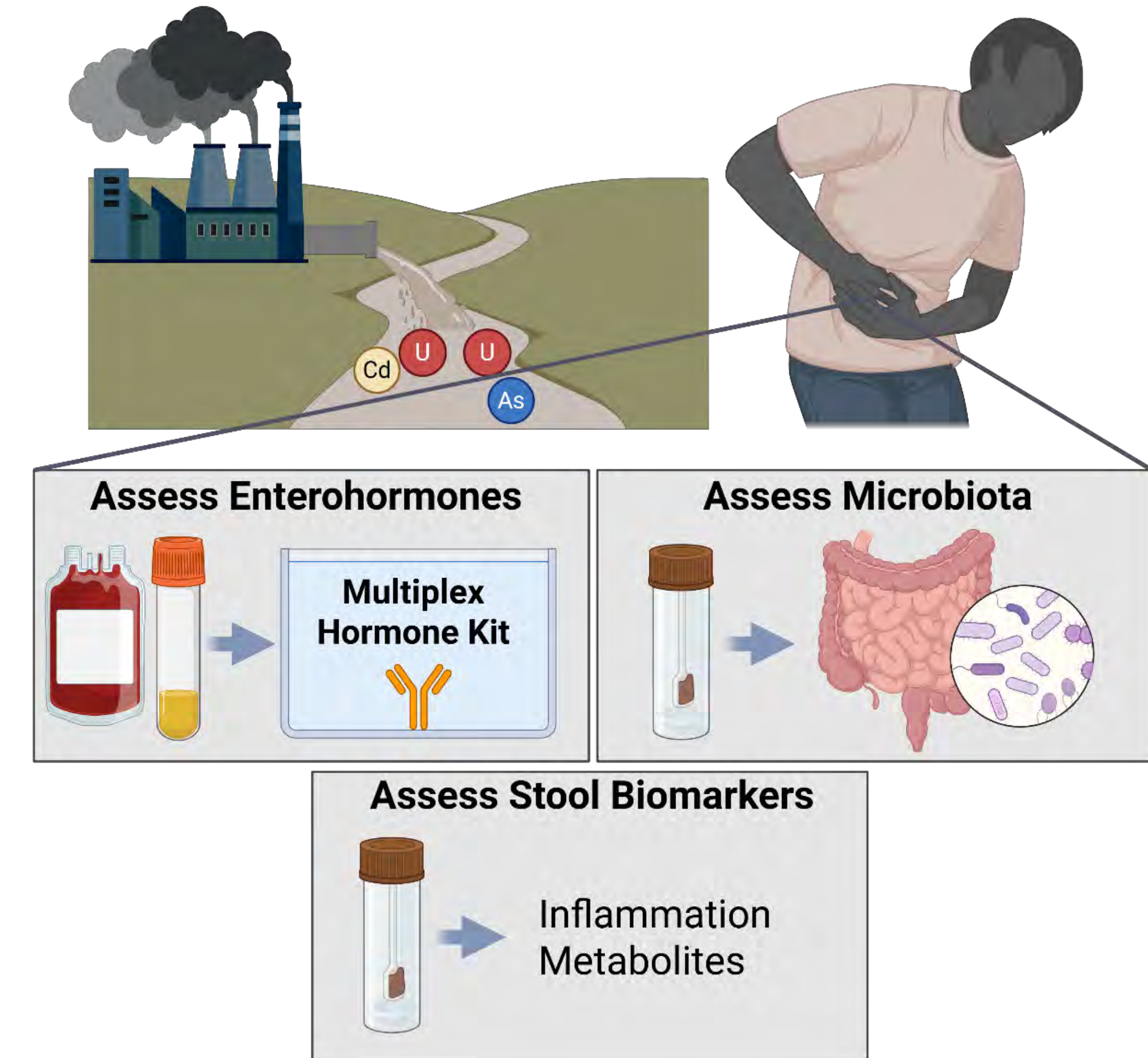
Uranium-Bearing Dust Instructs Intestinal Stem Cells to Become Enteroendocrine Cells: Using human intestinal organoids (mini-guts), we stimulated these mini-guts with uranium-bearing dust (UBD) that was collected from a church attic in Pagueate, NM – a village in the Laguna Pueblo situated adjacent to the Jackpile Mine, one of the largest open-pit uranium mines in the U.S. A) What we found was that the UBD caused intestinal stem cells in the mini-gut to become enteroendocrine cells. This was performed using technology called single-cell RNA sequencing and examining genes that are known to be enteroendocrine cell specific. B) We validated these findings by looking at hormones secreted by enteroendocrine cells, serotonin (5-HT) and PYY. UBD exposure increased the levels of 5-HT and PYY compared to unexposed mini-guts. *Atanga et al. 2024 Env. Health Perspect.*

Using animal models to understand the effects of heavy metals on the Gastrointestinal tract: Microbiota, Epithelium and Immune System



Using animal models to understand the effects of heavy metals on the microbiota: Animals are vital to research providing resource to study toxic particulates in an in vivo setting. A) Here we set-up an experiment where mice were provided water supplemented with uranyl acetate (UA), sodium arsenite (As) or the combination of UA and As for 45 days. Stool samples were collected at the beginning and the end of exposure to examine the gut microbiota and gut metabolites. B) Shows the Relative abundance of microbial species from the various groups. C) Metabolomics data showing tryptophan metabolism is associated with numerous gut microbes in male mice. D) Using murine intestinal organoids (mini-guts), we stimulated these mini-guts with UA. UA has been the standard form of uranium used in vitro and in vivo studies. This was done to determine if UA is similar to the UBD collected from the Laguna Pueblo. E) Interestingly, our assay to examine genes associated with different intestinal epithelial cell types shows UA does not have the same effect as the UBD. Therefore, more environmentally-relevant sources of uranium need to be utilized in our models. We are currently using water collected from the Rio Pagueate upstream and downstream of the Jackpile mine.

How can we understand the effects of heavy metal on the community?



Benefits to the Community

• Understanding Health Risks from Local Contamination

This research is helping to uncover how long-term exposure to uranium and arsenic from nearby mining activities may be affecting the health of people living in and around Laguna Pueblo - especially in the gut and immune system.

• Using Real Environmental Samples from the Community

Instead of using just lab-made chemicals, the study uses actual dust and water samples collected from places like the Rio Pagueate and the old Jackpile Mine. This makes the findings more accurate and directly relevant to the local environment and people's real exposures.

• Connecting Environmental Exposure to Real Health Markers

By testing stool and blood samples from community volunteers, the project is identifying early warning signs of inflammation or gut damage that might be caused by metal exposure - this can lead to earlier interventions and better health monitoring.

• Building the Case for Safer Living Conditions

The results can help community leaders, health advocates, and policymakers push for stronger cleanup efforts, better environmental protections, and more health services for affected families.

• Respecting and Including the Community in Science

The project involves Laguna Pueblo residents directly — not just as subjects, but as partners in understanding the impact of environmental injustice and helping to guide research that affects their future.

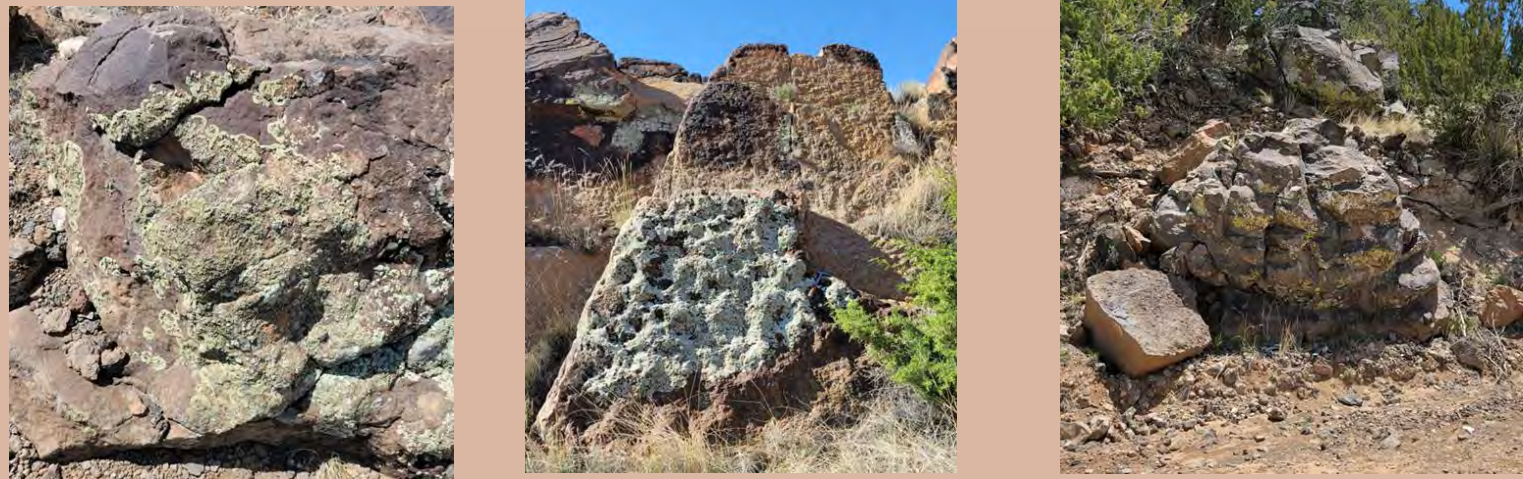
Tracing the Environmental Dust Release from the Jackpile-Paguate Mine, Pueblo of Laguna, NM by Particulate Matter Capture by Lichen

FREEMAN, Mackenzie¹, EL HAYEK, Eliane², CERRATO, Jose³, MACKEY, Tyler¹, ROROS, Andreanna¹, GARCIA, Amy⁴, JOJOLA, Gregory⁴ and SHUEY, Chris⁵, BREARLEY, Adrian J.¹

(1) Earth and Planetary Sciences, University of New Mexico, 221 Yale Blvd NE, Albuquerque, NM 87131, (2) COP Pharmaceutical Sciences, University of New Mexico, Albuquerque, NM 87106, (3) Civil Engineering, University of New Mexico, MSC 01 1070, Albuquerque, NM 87131, (4) Environmental and Natural Resources, Pueblo of Laguna, Paraje, NM 87007, (5) Southwest Research and Information Center, Albuquerque, NM 87106

Background

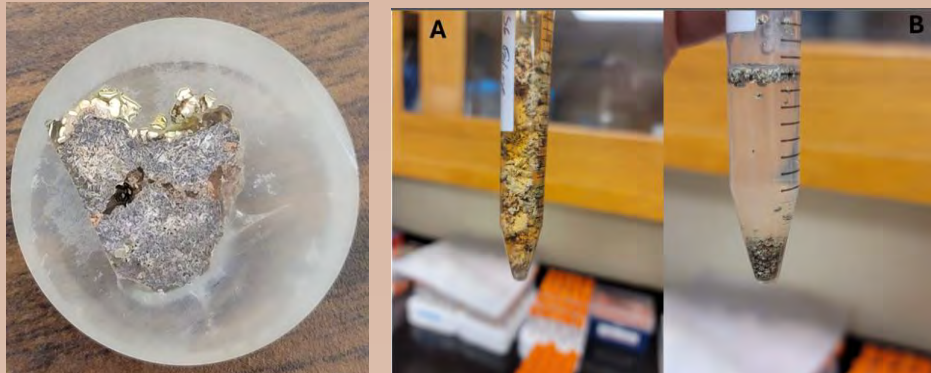
- The Jackpile-Paguate uranium mine located on the Pueblo of Laguna in the Grants mineral belt, NM was once one of the largest open pit mines in the world (2,656 acres of disturbed land).
 - Operation of the mine occurred from 1952 to 1982, and is no longer in operation.
 - Remediation began in 1989 and finished in 1995.
- Until recently there was no environmental air monitoring done in the area.
- Lichens were chosen for this to test their potential as a passive bio-monitor in a rural mining setting due to their long-lived nature, in order to understand legacy environmental air quality around the mine site and surrounding communities.



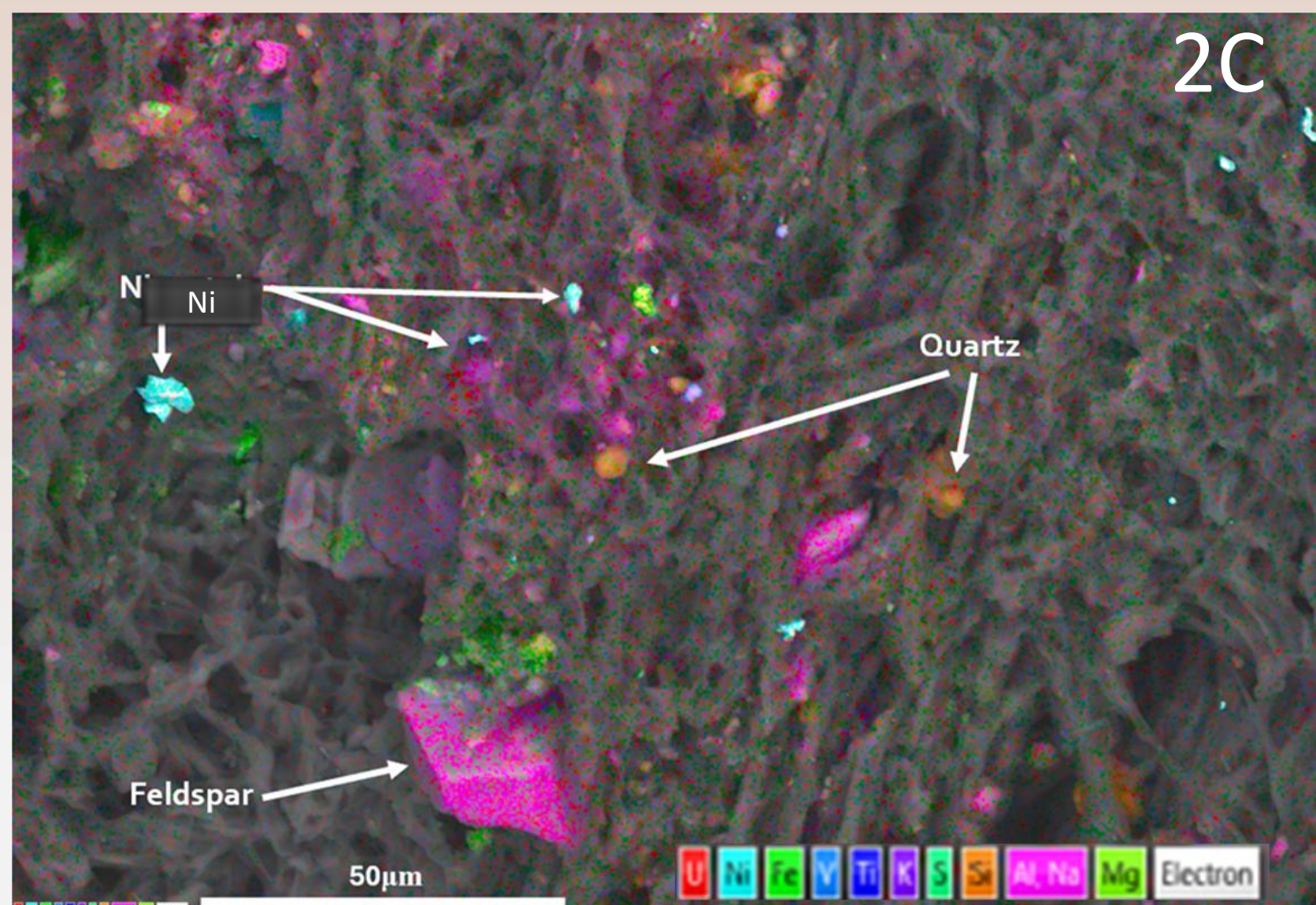
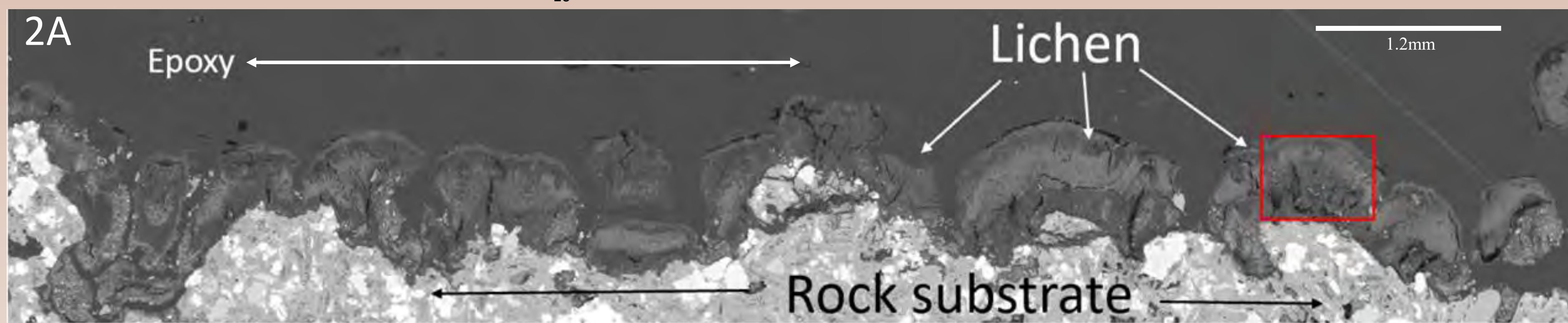
Lichen were collected from the sample sites from rocky outcrops, typically sandstone or basalt

Methods

- Field samples of lichen were analyzed as is (i.e. unwashed), and were either embedded in epoxy for SEM analysis, or removed from their substrate for ICP-MS analysis for particulate matter (PM) extraction.
- We developed a method for PM extraction for lichens, involving gentle removal of lichen samples from their substrate, sonicating the samples in DI water, pipetting out suspended particulates, and allowing evaporation, leaving behind extracted PM for analysis.
- ICP-MS analysis was performed on both lichens with included PM, and extracted PM to determine trace element compositions. (figure set 1)
- SEM analysis was performed on epoxy embedded lichen and extracted PM to determine mineralogical compositions of PM. (figure sets 2-4)
- SEM analysis focused on particles with high backscattered electron contrast (i.e. higher atomic number) and size ranges of PM₁₀ and smaller.

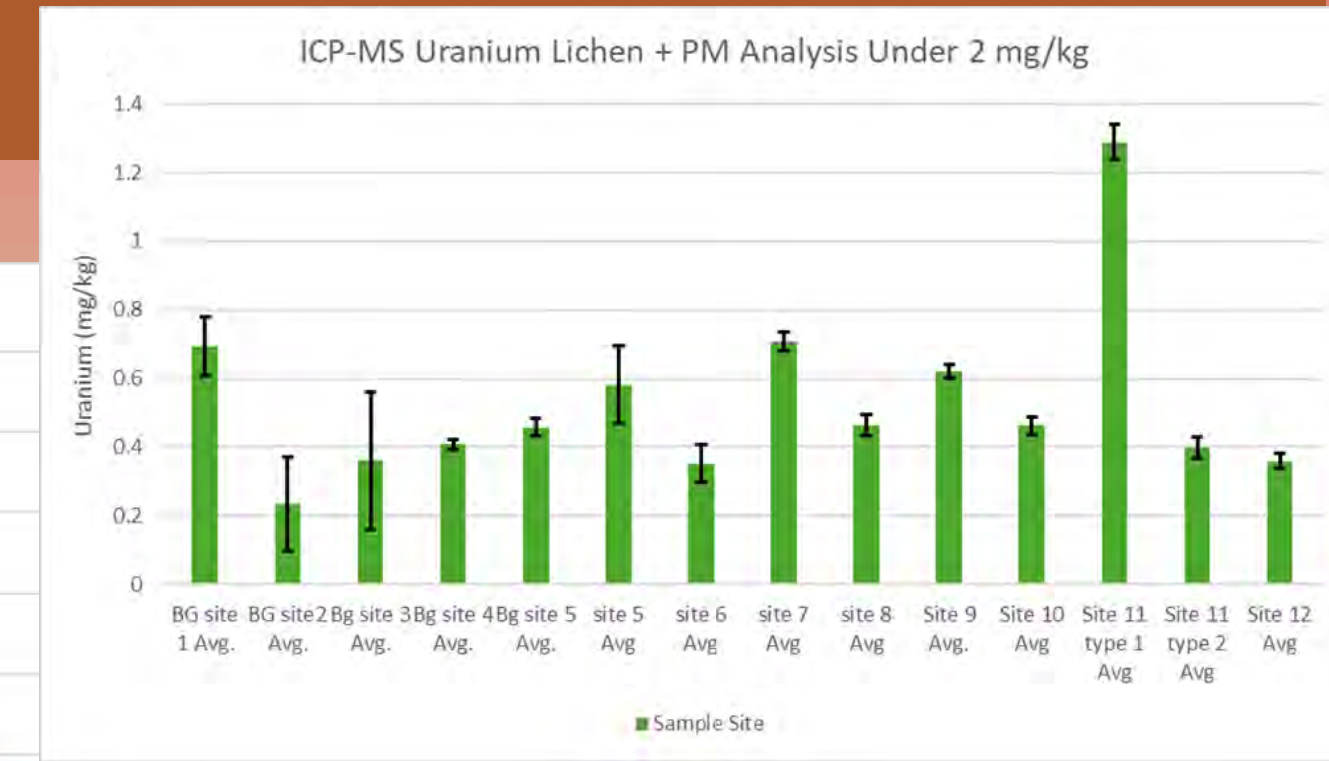
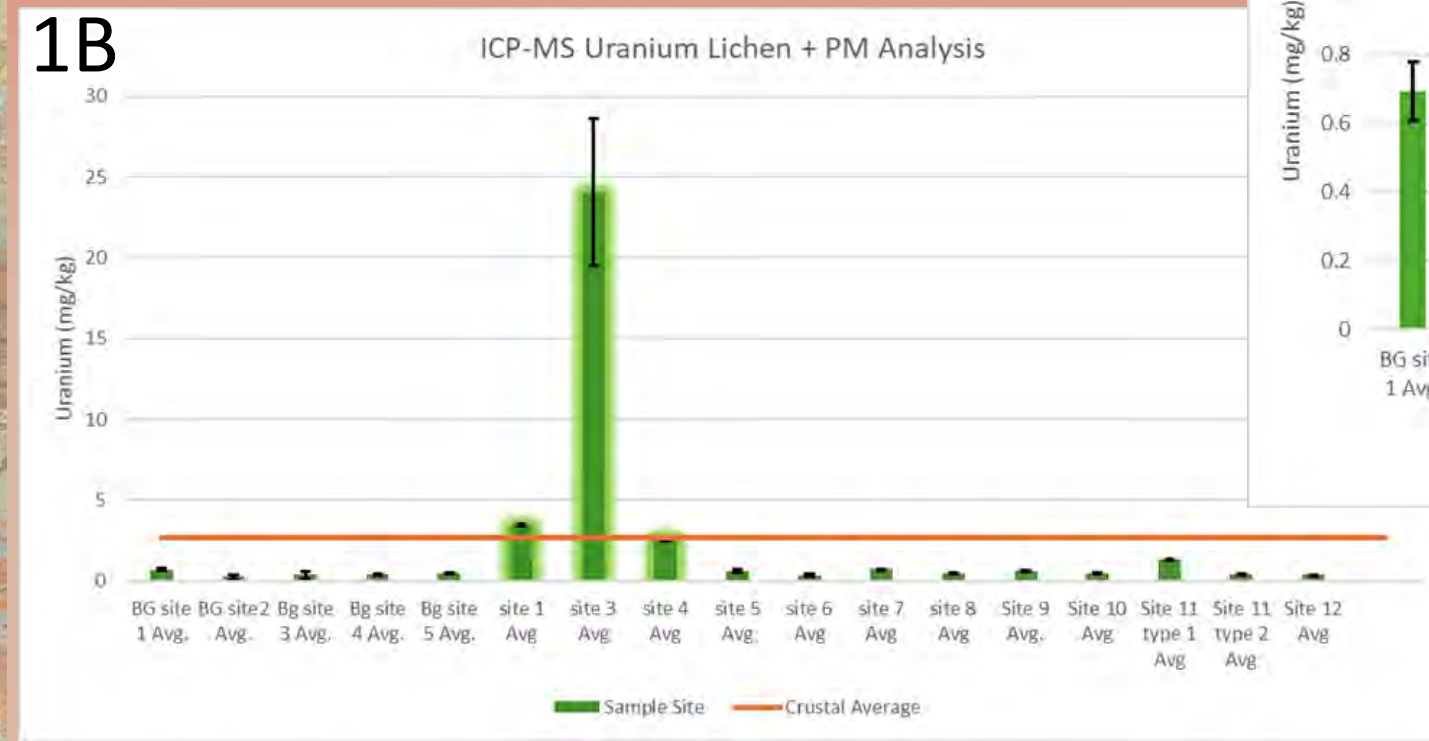
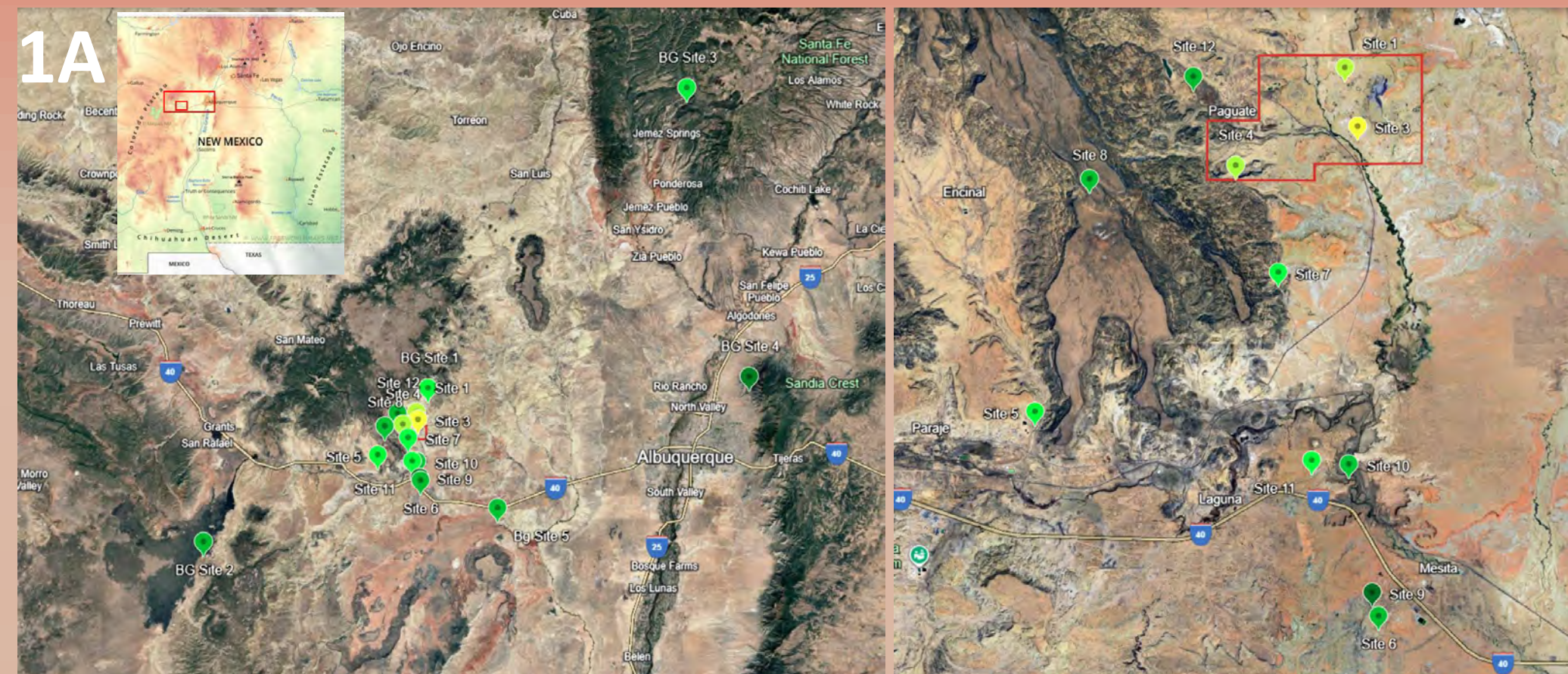


- Figure 2, a close up of the lichen cross section from site 2, clearly showing the tube-like structure of the lichen thalli, and how particles are entrapped during the lichen's lifespan as it grows.
- Trapped particles consist of feldspar, quartz, and clay minerals typical of aeolian transport in this region.
- Other particles, such as the Ni-enriched grains, are from anthropogenic sources, not likely related to the mine's dust output.

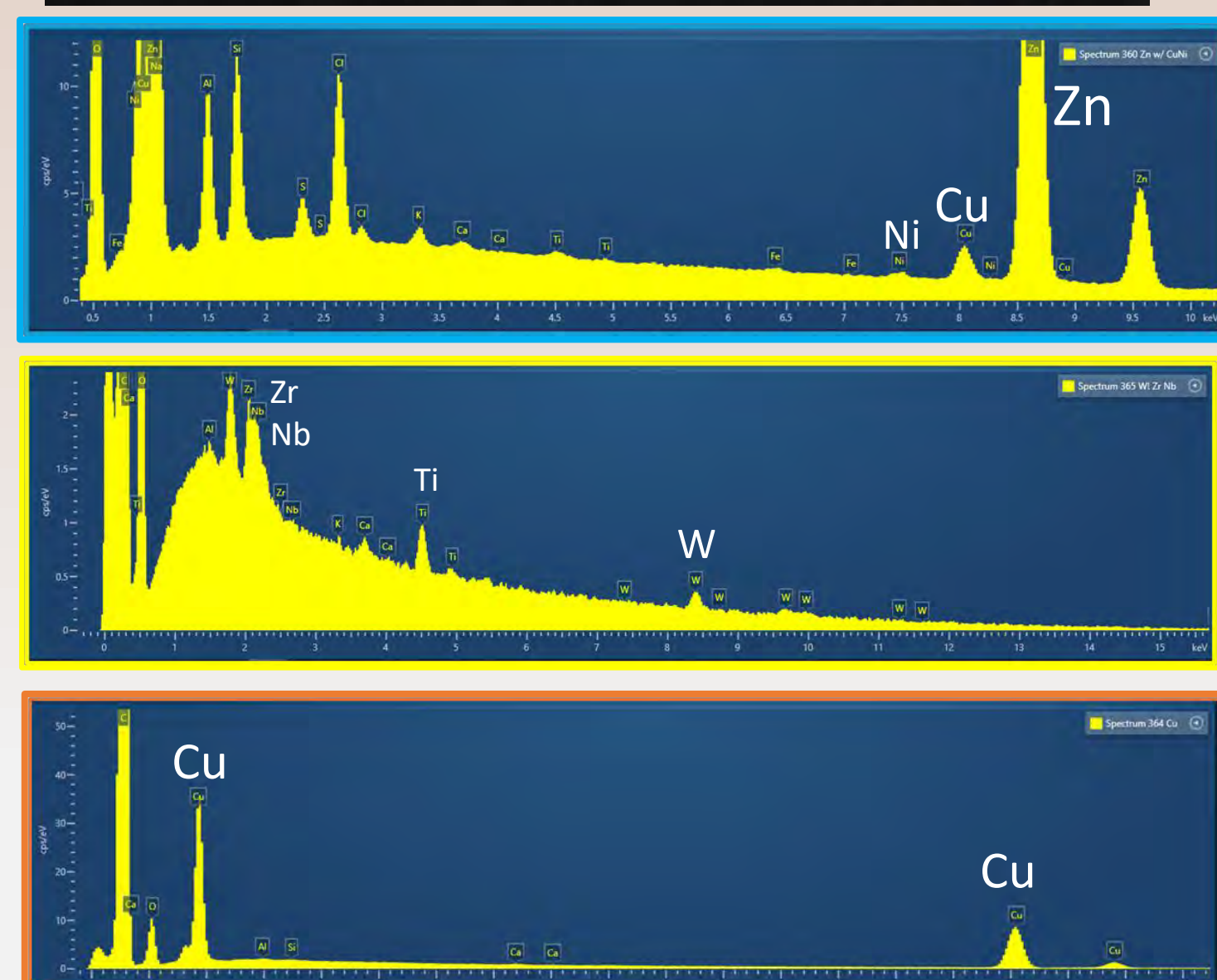
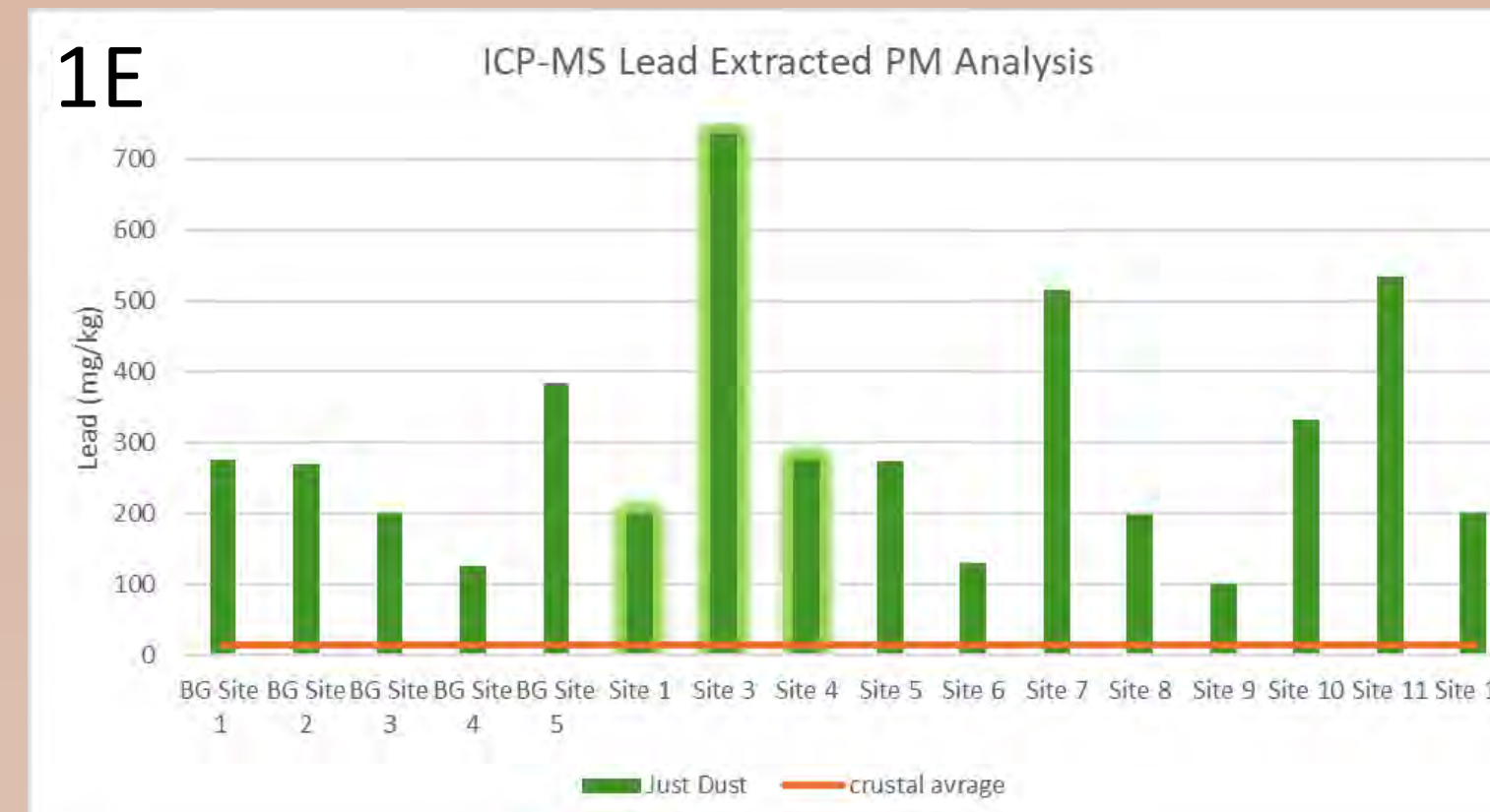
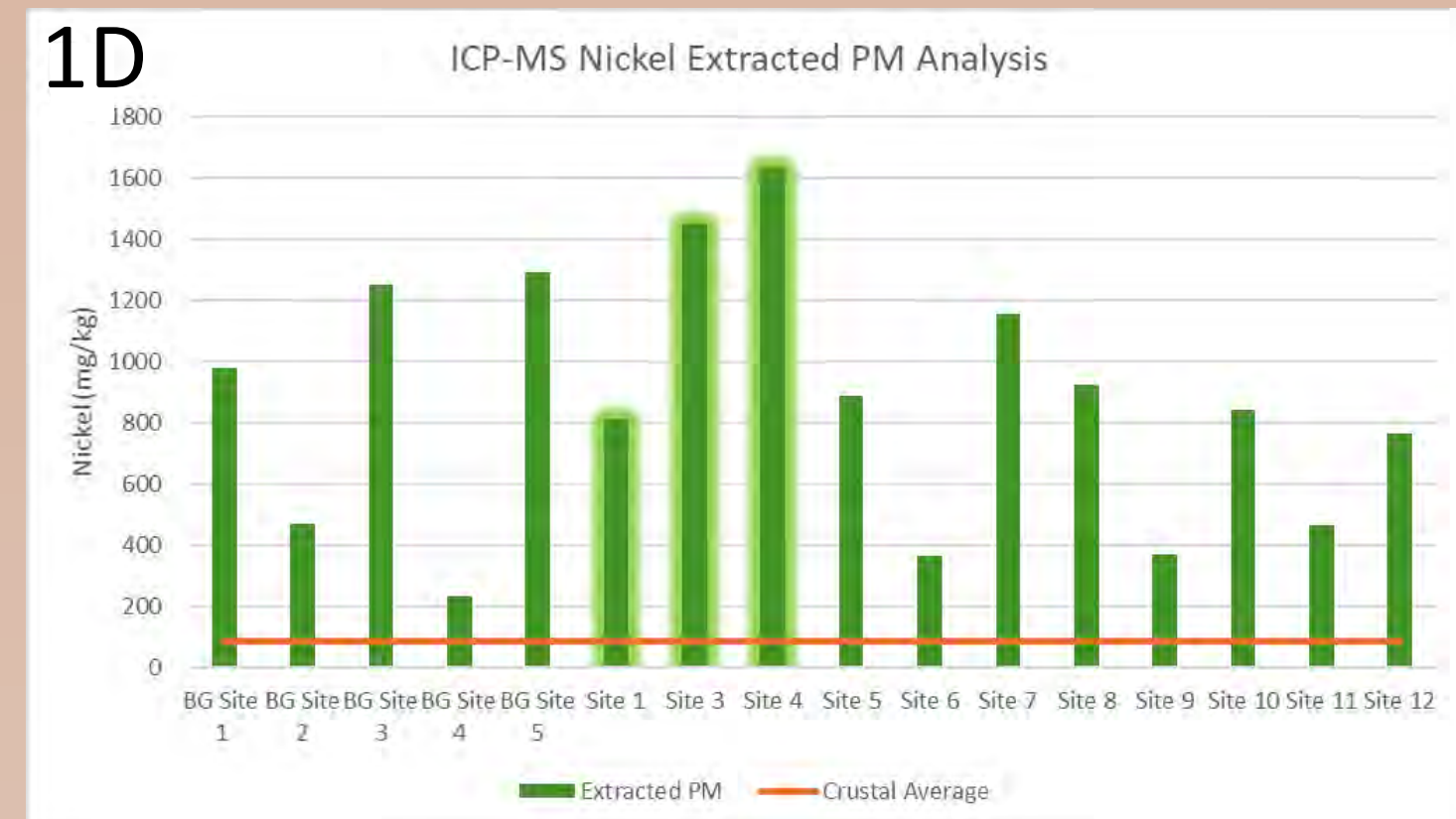
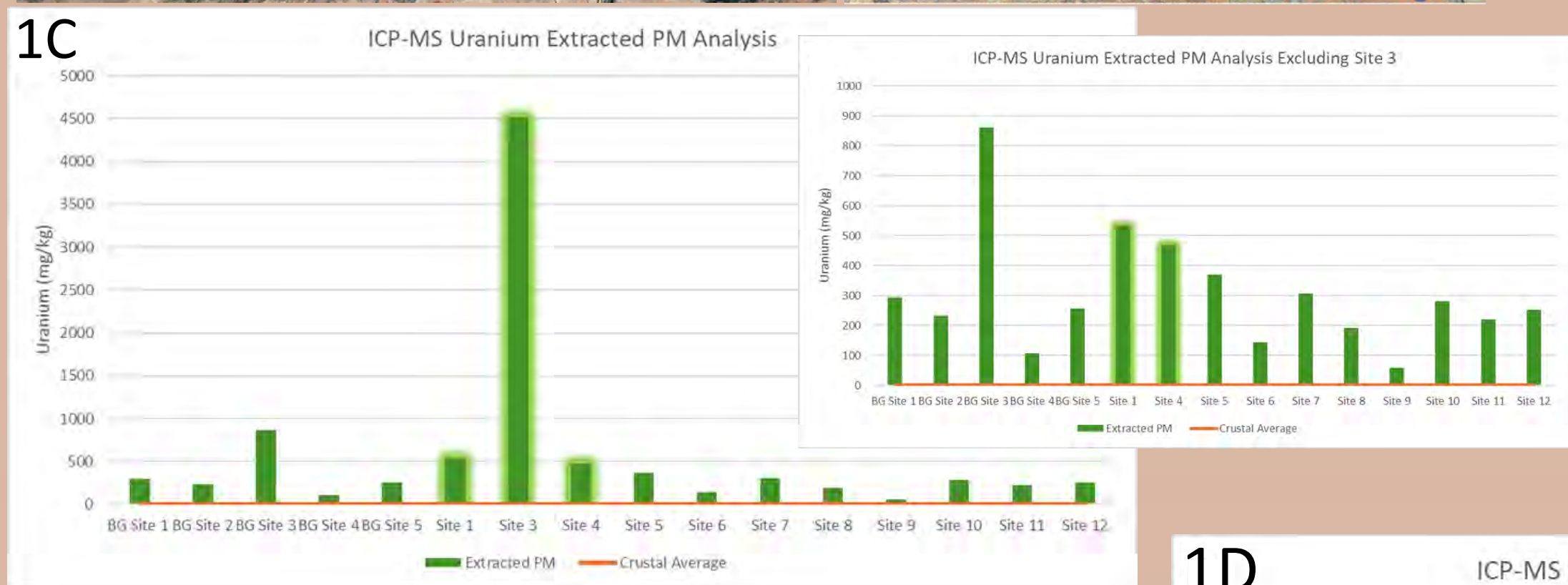


- 2A.) BSE cross section from site 2 of the embedded lichen on its substrate, showing the thallus structure.
- 2B.) High magnification image of a thalli, showing trapped particles and the Ca oxalate band produced by the lichen.
- 2C.) High magnification BSE image with an EDS elemental map overlay, showing particles with grain sizes of 1-25μm from both natural and anthropogenic sources.

Results



- Figure 1 shows a map of sample sites within the mine, surrounding communities, and more distal areas for background comparisons. The colors of the points correspond to U concentration at each site when comparing lichen + PM vs extracted PM.
- The highest concentrations of U exist within the mine site area, with concentrations falling sharply once in the surrounding areas outside of the mine.
- Uranium is highly enriched in the mine compared to sites within the surrounding communities.
- This observation does not directly apply to other heavy metals, whose concentrations are possibly influenced heavily by other anthropogenic inputs such as vehicular traffic from the nearby highway or industrial outputs from farther away.



- Figure set 1B-E are histograms of ICP-MS analysis concentrations for all sites for U both in the lichen + PM and extracted PM, as well as Ni and Pb for extracted PM. On all graphs the orange line represents the crustal average for the specified element, and the heavier green bars are sites that are within the mine boundaries.
- For U and Pb (figures 1B, C, and E) the highest concentrations are found at site 3, and are many times above the crustal average.
- These graphs show that lichen are able to accumulate high amounts of heavy metals as they grow, and when comparing the increase in U concentration between lichen + PM (1B) and extracted PM (1C), we see that lichens are seeming to keep these elements as particulates.

- Figures 3 and 4 are of extracted PM taken in SEM BSE mode at site 5 and site 4 respectively. Both figures display particles of anthropogenic origin, ranging from <2.5μm to 15μm in size.
- Figure 3 shows 3 alloy particles of different compositions. The particle circled in blue is a Zn alloy (with Cu and Ni). The particle circled in yellow, is composed of ZrNbTiW; and the particle circled in orange is Cu-rich.
- Figure 4 shows 2 particles attached to a large feldspar grain. The particle circled in orange is likely a stainless steel alloy and the particle circled in green is likely a Cu-rich alloy composed of CuFeCrTiMn.
- These particles are a small sample of a diverse range of metallic alloys found within the extracted PM. The majority of the particles have grain sizes in the range PM₁₀ to < PM_{2.5} that are of concern for inhalation exposures.

Acknowledgements

I would like to thank the Pueblo of Laguna and the Environmental and Natural Resources Department of the Pueblo of Laguna for allowing me access and permission to sample, making this project possible.

This study is funded by NIEHS P42 ES025589 with support from the UNM College of Pharmacy and the UNM Comprehensive Cancer Center