M WRRI Student Water Research Grant Progress Report Form

Progress Report due November 1, 2019
Draft Final Report due April 30, 2020
Final Report due May 31, 2020

1. Student Researcher: Michael Whiting
   Faculty Advisor: Dr. April Ulery

2. Project title:

   Monitoring Toxic Metal Uptake by Corn Grown in Agricultural Fields Across Animas and San Juan Rivers

3. Description of research problem and research objectives.

   During a study completed in 2018, three samples of corn kernels were found to exceed the World Health Organization's (WHO) limit of 0.05 ppm of lead (Pb) in corn (WHO/FAO, 2017). This study analyzed corn (*Zea mays*) samples from fields irrigated by the Animas and San Juan Rivers.

4. Description of methodology employed.

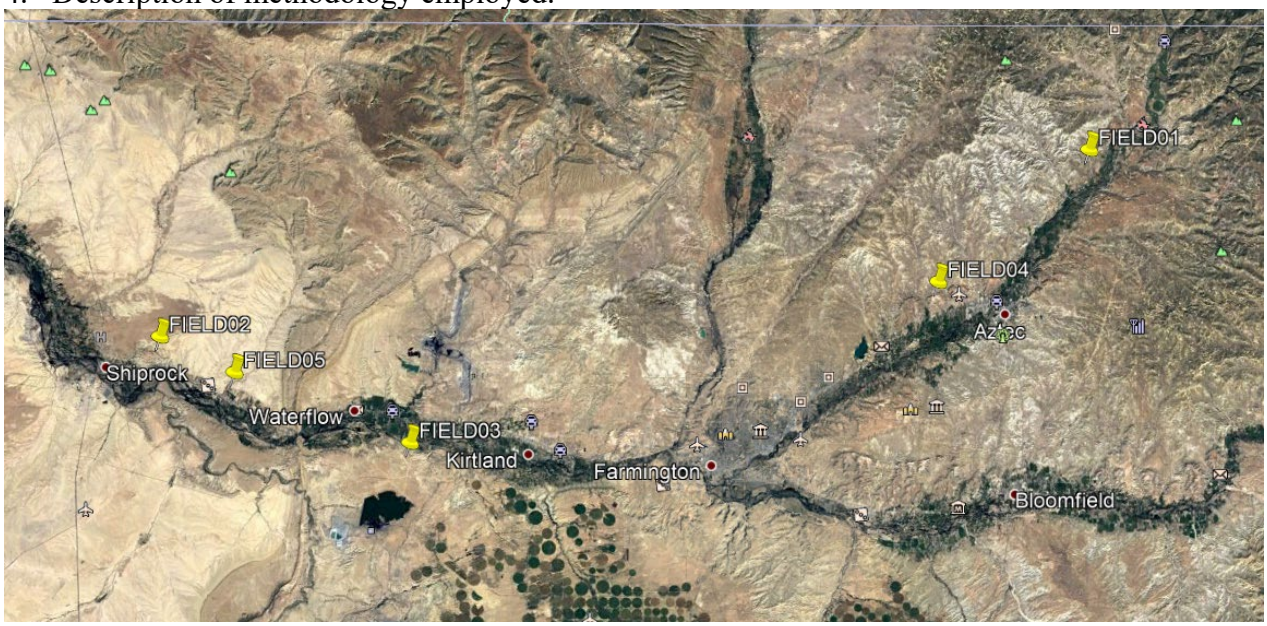

Figure 1. A Google Earth image with the approximate locations of the five sampling corn fields.

The locations of five sampled fields are shown in Figure 1. Initially, it was hoped that the same three fields from the previous study (Matthews, 2019; Matthews et al., 2020) would be available for re-sampling, along with one new field along the Animas River to gauge the spread of the potential lead contamination. Two of the same fields, one along the Animas River and one along the San Juan River, were available and were included in our study. However, one of the fields along the San Juan River was not being cultivated at the time of sampling for this study; so,

an alternative field along the San Juan River was identified. During the initial sampling trip to Farmington, an opportunity to sample a fifth farm became available, resulting in a total of five fields sampled (Figure 1). After land-owner consent, each field was divided into four sections starting closest to where the irrigation source entered the field, with three sample sites per section (e.g., Figure 2 shows the approximate sampling scheme in one field). The sample sites were chosen at random within each section. Farmers were responsible for their own seed; the seed was not provided by NMSU. Initial sampling locations were determined in the pre-growing season, prior to corn seed germination but after planting. Sites were recorded using a Global Position System (GPS) device (Garmin E-trax) and were sampled once during the pre-growing season for soil, again in the growing season for leaf tissue, and one last time in the harvest season for corn cobs (husk and kernels). Some sites were visited again late in the harvest season (December 2019) to sample roots from the corn plants. Farmer anonymity has been preserved by not listing any GPS coordinates in this report.

During the pre-growing season, June 2019, initial scans of the soil were taken using the Delta Premium 6000 portable X-ray fluorescence (PXRF) spectrometer (Olympus, Waltham, MA, USA) to analyze for total elemental concentration in the surface soil. Each scanned site was located using the same Garmin E-trax GPS device and soil samples were collected from the top 15 cm of the surface using a soil auger. For very loose, sandy soil, a small stainless-steel hand shovel was used to assist with the soil sample collection. Soil samples were placed in labeled plastic bags and then put into coolers for transport to New Mexico State University (NMSU) main campus (Las Cruces) for laboratory analysis. Upon arrival at NMSU, the soil samples were spread out to air dry in a designated area in the research laboratory. After three days, the air-dry soil was passed through a stainless-steel 2-mm sieve. The soil samples were microwave digested using method EPA 3051A, without hydrofluoric acid. Each digest used two blanks and two digestion vessels containing NIST Montana I 2710a or NIST Montana II 2711a for quality and control purposes. A random sample was chosen to be used as a duplicate for each digestion batch as well. The digests were analyzed for aluminum (Al) and lead (Pb) using Inductively Coupled Plasma - Optical Emission Spectrometry (ICP-OES) following EPA SW-846 Method 6010D (EPA, 2014). Arsenic (As) was analyzed using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) following EPA SW-846 Method 6020B (EPA, 2014).

Navigating back to the same soil sampling sites, in August 2019, during the growing season, fully mature leaf tissue samples were collected using a stainless-steel knife from the corn plants that grew at the GPS marked sites. Two leaves were taken per plant. As leaves were collected, each sampled plant was flagged using orange flagging tape for later reference and corn cob collection. The leaf tissue samples were placed in plastic bags and put in ice chests to prevent leaf decay during the <1 day transport back to Las Cruces. Once back at NMSU, each leaf tissue sample was washed using deionized (DI) water mixed with a small amount of phosphate-free soap (0.1 to 0.3% detergent solution) and rinsed thoroughly with DI water. The samples were then placed on new paper plates and put in a drying oven for two to three days at 65°C. The dried leaf tissue samples were ground using a commercial spice grinder, except for Field 1, which was ground using the Wiley Mill with a 0.85-mm screen. The ground samples were microwave digested using EPA Method 3052, without hydrofluoric acid, for plant material and analyzed using ICP-OES for aluminum and lead and using ICP-MS for arsenic.

Figure 2. A Google Earth image showing the locations of the sampling sites at Field 1. The samples were labeled for each field using the following notation: first number (1-12) represents the number of total samples in the field and as a sample designator. The second letter and number combination represent the section in which the sample was collected. The Black outline represents approximately where the corn plants were being cultivated at the time of sampling, blue arrows represent the path of the irrigation water.

Returning to the earlier plant sampling sites, during the harvest season in September 2019, corn cobs with kernels were collected using the recorded GPS coordinates and the orange flagging tape applied earlier during the growing season. Corn cobs were collected using a stainless-steel knife and placed in labeled plastic bags. These bags were kept in a refrigerator at

approximately 4ºC before being placed in an ice chest for transport to NMSU within 24 hours. At NMSU, each corn cob was processed by removing the husk from the cob, washing separately in DI water with a small amount (0.1 to 0.3% detergent solution) of phosphate-free soap and rinsing thoroughly in DI water, then the husks were placed in the drying oven at 65°C for two to three days. The remaining corn cob material underwent the same steps, but the kernels were removed from the cob using a stainless-steel knife and an acrylic cutting board before being placed in a new labeled paper bag and dried in the oven at 65°C. The corn husks and kernels were ground using a Wiley Mill with a 0.85-mm screen. Field 1 corn husks were ground using the same spice grinder used to grind leaf tissue from fields 2-5. During the study, it was suspected and later verified that the Wiley Mill was contributing additional lead during the grinding process. The contaminated corn kernel results have been omitted because we do not trust the data. Corn kernels and husks were microwave digested using EPA Method 3052, without hydrofluoric acid, for plant material. As with the leaf tissue, ICP-MS was used to analyze for arsenic and ICP-OES was used to analyze for aluminum and lead. All of the procedures are summarized in Figure 3.

Additional root samples were collected from Field 1, Field 2, and Field 3 to compare to the questionable lead concentrations discovered in the corn kernels. This was a modification to the original project design and was decided upon after the discovery of the unexpected lead concentrations found in two fields. The intent behind the analysis was to explore possible entryways of lead into the plants. Sampling from the same previously sampled plants was a high priority; however, some fields were unable to be correspondingly sampled due to wildlife digging the remaining roots up at the end of the harvesting season. Root samples were still collected but they do not represent the same individual plants sampled throughout the study. Roots were processed similar to aboveground plant tissue and analyzed for aluminum and lead by ICP-OES. It is important to note that it is nearly impossible to completely remove all soil from the root structures and it is possible some soil was analyzed along with the root material. Previous studies have found that heavy metals such as lead may accumulate on, or in, the roots of various plants (Khan et al., 2015; Pourrut et al., 2011).

5. Description of results; include findings, conclusions, and recommendations for further research.

Delay in Analysis: During the study, the ICP-OES instrument used to analyze our samples was inoperable for several months and required the manufacturer to send a technician to repair the unit. This pause in analysis in the laboratory created a large backlog of samples needing to be analyzed on the ICP-OES. The ICP-OES has since been repaired and samples were analyzed. A potential source of lead contamination was identified in the lab processing equipment rendering some of the samples unreliable, including all corn kernels. The equipment has since been removed from the lab.

Initial PXRF results did not exceed screening level toxicity limits in residential soil for arsenic and lead (Tables 1 and 2; note that PXRF was not used for aluminum). Soil samples that were collected and analyzed using ICP-OES and ICP-MS support the PXRF results for Fields 1, 4, and 5 and also verified that aluminum was below guideline limits in the soils of all five fields analyzed (Table 3). Two fields, Field 2 and 3, exceeded the SSL of 7.07 ppm for arsenic measured on the ICP-MS, but arsenic did not accumulate in the plant tissue (Table 4).

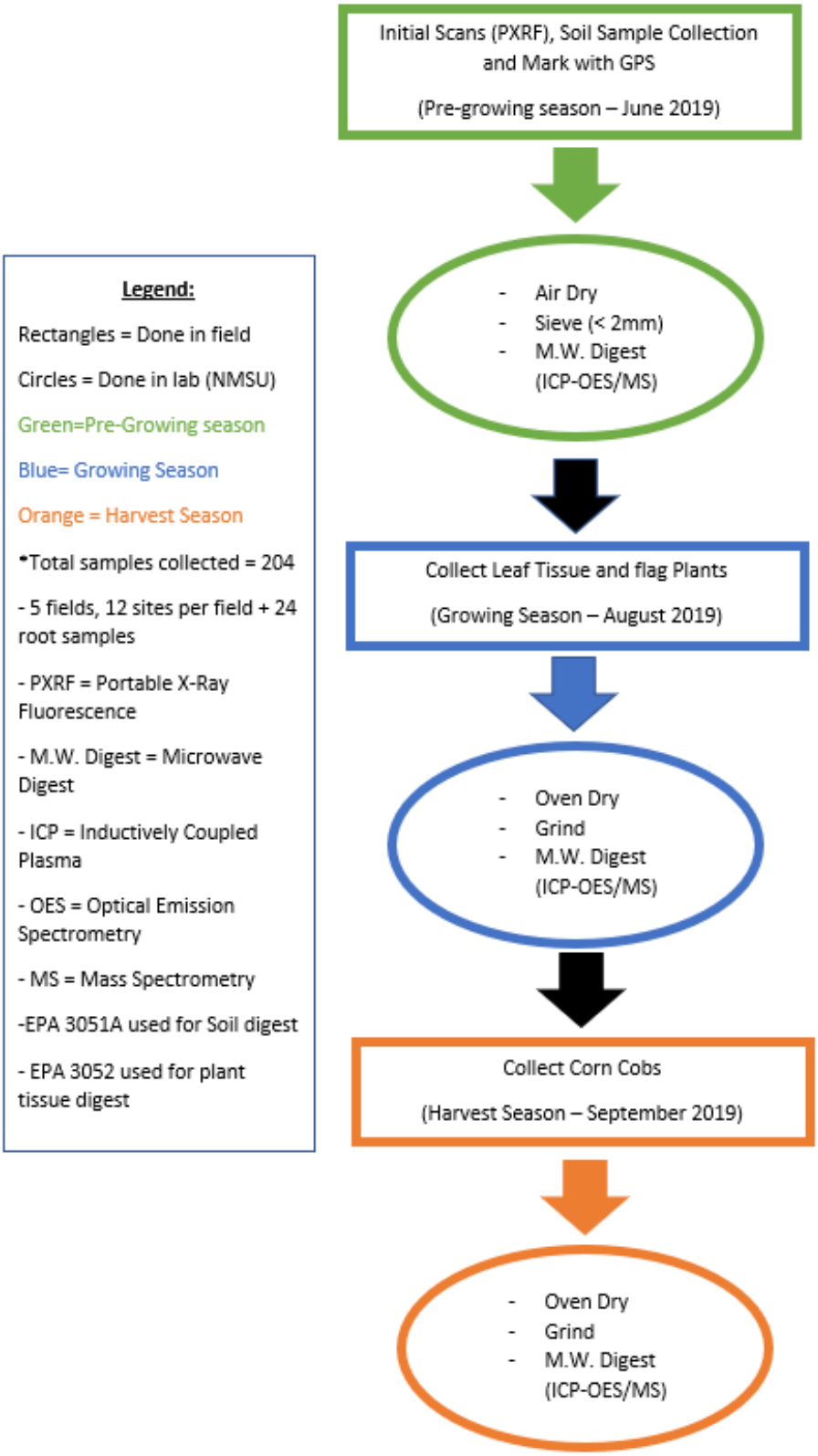

Figure 2. A flow chart summarizing the collection methods and analyses procedures used in this study.

Table 1. Descriptive statistical parameters determined by SAS, n=12, for Fields 1-5, soils irrigated by San Juan River and Animas River water arsenic concentrations. See Figure 1 for approximate location.

Field	Mean	StdDev	Std Error	Mean	StdDev	Std Error	Mean	StdDev	Std Error
Soil Arsenic (mg/kg)									
ICP Pre-planting				PXRF Pre-planting			PXRF Post harvest		
1	4.22	1.11	0.32	3.13	2.34	0.68	3.19	2	0.58
2	16.54	3.33	0.96	5.44	1.76	0.51	4.26	0.98	0.28
3	12.54	1.69	0.49	3.93	1.89	0.55	3.34	1.2	0.35
4	4.63	0.75	0.22	3.74	2.16	0.62	1.43	1.47	0.43
5	4.92	0.28	0.08	3.34	1.83	0.53	4.43	1.58	0.46

- "PXRF-Pre" refers to scanning completed in June, 2019. "PXRF-Post" refers to scanning completed post growing season, winter 2019-2020. All values are in ppm (mg/kg).

Table 2. Descriptive statistical parameters determined by SAS, n=12, for Fields 1-5, soils irrigated by Animas River and San Juan River water lead concentrations. See Figure 1 for approximate location.

Field	Mean	StdDev	Std Error	Mean	StdDev	Std Error	Mean	StdDev	Std Error
Soil Lead (mg/kg)									
ICP Pre-planting				PXRF Pre-planting			PXRF Post harvest		
1	61.63	25.19	7.27	51.98	12.32	3.56	47.55	15.74	4.54
2	35.18	5.18	1.49	32.42	4.23	1.22	27.43	4.14	1.19
3	37.85	8.57	2.47	31.17	6.93	2	25.23	5.8	1.68
4	58.08	20.75	5.99	61.52	26.67	7.7	97.53	19.09	5.51
5	14.49	1.77	0.51	15.04	1.89	0.55	13.97	2.8	0.81

- "pre-planting" refers to soil collected and PXRF scanning completed in June, 2019. "Post-harvest" refers to PXRF scanning completed post growing season, winter 2019-2020 at approximately the same locations in each field. All values are in mg/kg (ppm).

Leaf tissue samples from the five fields were analyzed using ICP-OES and ICP-MS and all had arsenic concentrations below WHO levels of toxicity for human consumption (Table 4). Aluminum concentrations in the leaf tissue did exceed the WHO level for edible produce (table 3), however, corn leaves are not commonly consumed by humans. The aluminum and arsenic concentrations in the corn husks were both below the WHO maximum level for edible produce, (Tables 3 and 4). Leaf tissue in Fields 1, 4, and 5 and corn husks from all the fields (Table 5) exceeded the WHO guidelines of 0.05 ppm for lead; however, that limit was proposed for edible corn and not leaves or corn husks. During the study, it was noted that wildlife, including livestock, do consume the various other parts of the crops, including the corn leaves and husks. Arsenic and aluminum remained below the Maximum Allowable Limits (MAL) set by the WHO in all five fields (Table 6). In reviewing plant lead concentrations (Table 5), it was realized that 4 out of 5 fields followed a similar trend in the amount of lead in various plant parts. However, one

field was reversed in this trend, and it had been processed differently than the others, leading the team to suspect, and later verify, that a piece of lab equipment was causing lead contamination.

Table 3. Soil and plant tissue Al concentration descriptive statistical parameters determined by SAS, n=12, for Fields 1-5, soils irrigated by San Juan River and Animas River water aluminum concentrations. See Figure 1 for approximate location. All samples analyzed on ICP-OES.

Field	Mean	StdDev	Std Error	Mean	StdDev	Std Error	Mean	StdDev	Std Error
Aluminum in soil and plant tissue (mg/kg)									
ICP Pre-planting (Soil)				Leaf Tissue			Corn Husk		
1	13516	5396	1558	28.49	16.01	4.62	6.15	5.4	1.56
2	14798	2920	843	92.65	89.08	25.71	8.14	9.19	2.65
3	15087	3499	1010	27.03	9.74	2.81	13.03	7.93	2.29
4	10979	1268	366	82.77	26.84	7.75	6.48	5.53	1.6
5	11936	2482	717	68.67	24.28	7.01	5.55	19.14	5.52

-All values are in ppm (mg/kg).

Table 4. Descriptive statistical parameters determined by SAS, n=12, for Fields 1-5, leaf tissue and corn husks arsenic concentrations. See Figure 1 for approximate location

Field	Mean	StdDev	Std Error	Mean	StdDev	Std Error
Arsenic in plant tissue (mg/kg)						
Leaf Tissue				Corn Husk		
1	0.16	0.11	0.03	0.02	0.01	0
2	0.08	0.03	0.01	0.01	0.01	0
3	0.09	0.04	0.01	0.01	0.03	0.01
4	0.4	0.17	0.05	0.02	0.02	0.01
5	0.13	0.05	0.02	0.01	0.02	0.01

-All values are in ppm (mg/kg).

Table 5. Descriptive statistical parameters determined by SAS, n=12, for Fields 1-5, leaf tissue and corn husks lead concentrations. For corn roots, n=12 for Field 1, n=7 for Field 2, and n=5 for Field 3 root samples. See Figure 1 for approximate location.

Field	Mean	StdDev	Std Error	Mean	StdDev	Std Error	Mean	StdDev	Std Error
Lead in plant tissue (mg/kg)									
	Leaf Tissue			Corn Husk			Corn Roots		
1	0.86	1.64	0.47	0.36	1.23	0.36	1.29	2.19	0.83
2	0.01	0	0	5.4	3.2	0.92	4.93	4.63	1.34
3	0.01	0	0	9.73	5.26	1.52	0.01	0	0
4	2.68	2.8	0.81	10.82	7.45	2.15	-	-	-
5	6.58	9.45	2.73	8.33	2.94	0.85	-	-	-

- All values are in ppm (mg/kg).

The research team conducted a follow-up investigation of field soils and plant roots to determine lead patterns in the field. After returning to the Farmington area, post-growing PXRF scans of the soil were made along with the collection of random root samples left in-the-ground but not taken as part of the original plant sampling plan. Generally, the literature reports that most plants grown in contaminated soil have the highest metal concentration in the roots, *decreasing* with distance up the plant (Khan et al., 2015).

Average lead, aluminum and arsenic soil levels measured by PXRF were below EPA and NMED limits regardless of when the scans were taken. Two fields, Fields 2 and 3, did exceed the NMED limit for arsenic when analyzed using ICP-OES. The concentrations were not unexpected given the high natural occurrence of arsenic bearing minerals in New Mexico (Dunbar et al., 2002) and previous research results from the area (Fullen, 2017; Jha et al., 2021). Roots collected from Field 1 and Field 2 both had average concentrations of lead higher than the WHO guideline for consuming fruiting vegetables (even though people do not typically eat corn roots). Roots grown in Field 3 all had lead levels below the detection limit of the ICP-OES (0.007 ppm) (Table 5). Table 6 summarizes the different analyses completed on the samples and lists the soil screening limit/maximum allowable limits and the method detection limits.

Table 6. Type of analysis conducted for each element depending on where the element is located, either as part of the plant tissue or in the soil. The SSL (mg/kg) and MAL (mg/kg) are also listed along with the MDL (ppm) for each method.

Element	As (Soil)	Pb (Soil)	Al (Soil)	As (plant tissue)	Al (Plant tissue)	Pb (Plant tissue)
Analysis	PXRF	PXRF	N/A	ICP-MS	ICP-OES	ICP-OES
SSL(Soil Screening Limit) and Maximum allowable limits (MAL)	7.07[2]	400[3]	N/A	0.192 mg/d for a female weighing 63.5 kg[1]	18.29 mg/d for a female weighing 63.5 kg[1]	0.05
MDL (Method Detection Limit)	1-3	1-4	N/A	0.00007	0.024	0.007

-[1]MAL for As and Al plant tissue were calculated using the WHO/FAO codex for edible vegetables/fruits. Values are given in an amount per kilogram of body weight in the codex, so the limits were converted into an allowable amount per day. (WHO/FAO, 2017)
SSL=Soil screening limits set by [2.] New Mexico Environment Department (NMED) for As and Al, [3.]U.S. Environment Protection agency (EPA) for Pb. Values are in mg/kg (ppm).

Based on the literature, the lead concentrations observed in the roots do not support the hypothesis that the plants were extracting lead at high enough rates and amounts to store lead in the kernels. However, a subsequent project focusing on the bioavailability of lead in the soil is strongly recommended. Sequential extraction is a commonly used method that uses increasingly stronger reagents to extract lead from soil samples. This simulates different natural pH environments that can influence the uptake of lead into the corn plants. Employing this method would help researchers determine if high levels of lead are able to be transferred from the soil and into the edible portions of fruiting vegetable plants, such as corn. It is also recommended that the follow up project selects fields to represent the previous study while expanding to new fields to monitor lead in agricultural fields grown along the Animas and San Juan Rivers.

6. Provide a paragraph on who will benefit from your research results. Include any water agency that could use your results.

   - Local farmers and growers using irrigation water from the Animas and San Juan Rivers would need this project's results. These farmers and growers include members of the Navajo Nation and the citizens of Farmington and Aztec as well as other small communities in the watershed. The results will inform the farmers and consumers on crop safety and potential metal contamination and indicate the need for further testing. The research conducted could also benefit future students doing research in the region and, potentially help with understanding and predicting the effects of other mine spills. The results may also serve to inform the Water & Wastewater Utilities of Farmington and Aztec, NM about metal concentrations in corn being irrigated by the two rivers downstream of their Wastewater Treatment Plant outflows.

7. Describe how you have spent your grant funds. Also provide your budget balance and how you will use any remaining funds. If you anticipate any funds remaining after May 31, 2020, please contact Carolina Mijares immediately. (575-646-7991; mijares@nmsu.edu)

   - The current balance is $0.00. $2007.70 has been used for travel to and from Farmington and Las Cruces, NM. $2,190.32 has been used for lab supplies and $2,227.69 was used for analysis by ICP-OES. $24.40 was used for miscellaneous supplies such as flagging tape, coolers for transportation, and batteries for GPS device. $40.00 was used for poster printing to present at the 64th WRRI Conference.

8. List presentations you have made related to the project.
   - Presented poster at 64th WRRI Conference:
   - Presented poster at 2020 Animas and San Juan Watersheds Conference

9. List publications or reports, if any, that you are preparing. For all publications/reports and posters resulting from this award, please attribute the funding to NM WRRI and the New Mexico State Legislature by including the account number: NMWRRI-SG-2019.

   - Master of Science thesis in Plant & Environmental Sciences Department will be one of the products of this grant.

10. List any other students or faculty members who have assisted you with your project.

    - Gaurav Jha, Ph.D., University of California, Davis, Davis California
    - Barbara Hunter, ICP-OES/MS Analyst, New Mexico State University, Las Cruces
    - Kevin Lombard, New Mexico State University, Superintendent Farmington Agricultural Science Center (ASC)
    - Brandon Francis, New Mexico State University, Farmington ASC Staff
    - Jaime Grijalva, Lab Assistant, New Mexico State University, Las Cruces
    - Kaitlin Marry, Lab Assistant, New Mexico State University, Las Cruces

11. Provide special recognition awards or notable achievements as a result of the research including any publicity such as newspaper articles, or similar.

N/A

12. Provide information on degree completion and future career plans. Funding for student grants comes from the New Mexico Legislature and legislators are interested in whether recipients of these grants go on to complete academic degrees and work in a water-related field in New Mexico or elsewhere.

    -I am a graduate student and am expecting to graduate in July, 2021. My future career plans are to gain employment as an environmental scientist working in soil science. This project has demonstrated the importance of environmental science and I wish to continue to work within the field.

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