# NM WRRI Student Water Research Grant Progress Report Form
**Final Report due May 31, 2021**

1. Student Researcher: Bianca Wright
   Faculty Advisors: Dr. April Ulery, Dr. Kevin Lombard

2. Project title: Evaluating Soil Lead Bioavailability in Agriculture Fields Across Animas Watershed

3. Description of research problem and research objectives.

   A 2019-2020 study monitoring lead (Pb), arsenic (As) and aluminum (Al) in corn grown in the San Juan/Animas watershed found possible contamination of Pb in corn kernels (Matthews et al., 2020). The objective of this study is to verify elevated levels of Pb measured during the previous study and to determine possible pathway(s) of Pb accumulation in corn kernels.

4. Description of methodology employed.

   Two fields from the previous study were re-sampled to compare to previous data (Whiting et al., 2020). Two additional fields were evaluated using Portable X-Ray Fluorescence (PXRF) data to estimate the extent of possible soil Pb contamination in the watershed. Each field was divided into four sections each containing three sampling sites marked via GPS (Global Positioning System), resulting in a total of 48 individual sampling points in the watershed.

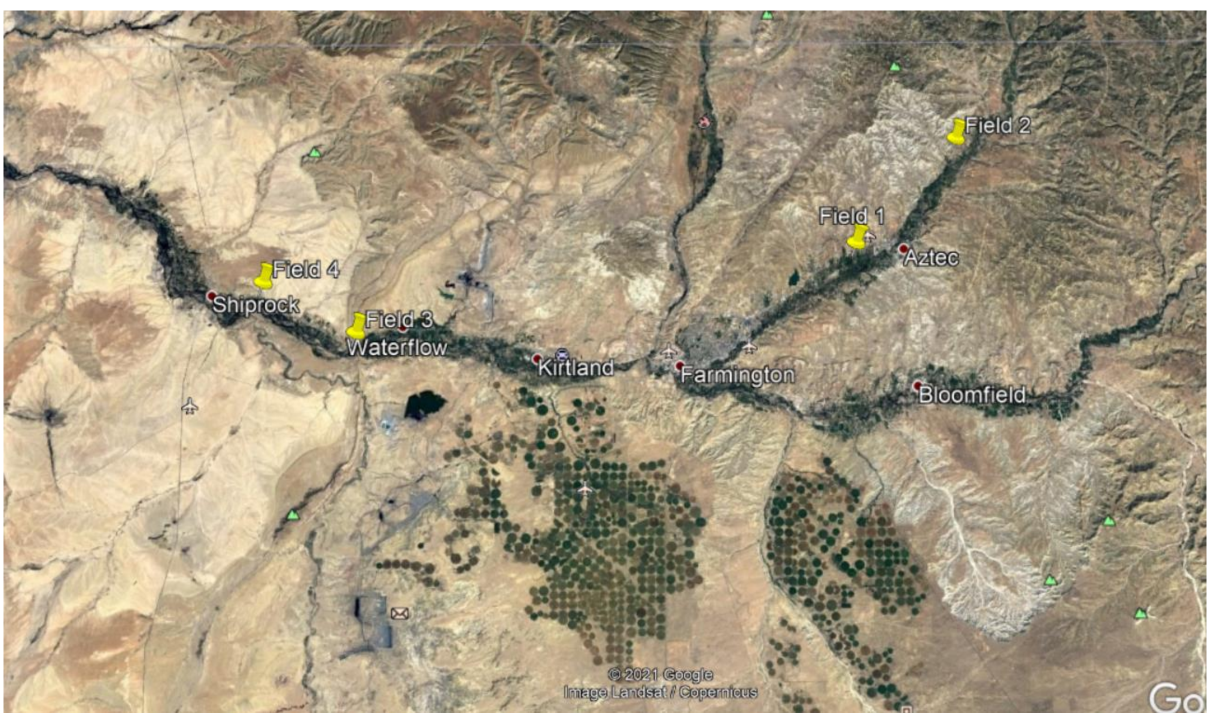


*Figure 1. A Google Earth image with the approximate locations of the four sampling corn fields. Fields 2 and 4 were also sampled during the 2019 study (Whiting et al., 2020).*

At each of the 48 sampling sites, whole corn plant samples (including roots, stalk, leaves, and corn cobs, as well as soil around the roots) were collected and placed in labeled nylon sacks. The samples were kept in a refrigerated building and vehicle during short-term storage and transport from Farmington, NM to New Mexico State University in Las Cruces, NM.

Once in Las Cruces, samples were placed in a cold room (~4°C) for storage as the various plant parts were removed and processed for total metal analysis using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). Soil surrounding the root balls was collected by gently shaking the plant over a clean plate designated and labeled for collecting the soil. The soil was air dried and sieved through a 2-mm stainless-steel mesh screen. Roots were removed from the aboveground portion of the plant using stainless-steel gardening pruners. The separated roots were placed in labeled paper bags and dried in an oven at 65°C for two to three days before being ground in a commercial blender (*Oster Duralast classic)*. The two uppermost, fully mature leaves from each plant were removed using the same stainless-steel pruners and placed in labeled paper bags and put in the drying oven at 65°C for two to three days to fully dry. After drying, the leaf samples were ground in the same commercial blender used to grind the root samples.

The corn cobs required additional processing prior to digestion and ICP-OES analysis. Corn cobs were removed from the cornstalk using stainless-steel pruners. Husk material was separated from the kernels by hand and each plant component was quickly washed using 0.1 to 0.3% detergent solution of phosphate-free soap and rinsed using pure deionized (DI) water. The corn husks were then placed in labeled paper bags and dried at 65°C for two to three days. Corn kernels were separated from the cob using a stainless-steel knife and an acrylic cutting board. The separated kernels were placed in labeled crucibles and allowed to dry in the oven for two to three days at 65°C. The dried materials were removed, and the corn husks were ground using the commercial blender used for previous plant material. Corn kernels from three of the fields were ground using a Wiley Mill. However, after the discovery that the Wiley Mill (now over 40 years old) might be a source of Pb contamination, the corn kernels from the remaining field were split into two samples – one half left whole, and the other half ground in the Oster *Duraclast* commercial blender used to grind the other plant parts. The Wiley Mill has since been removed from use in the Soil Science Research Lab.

Dried samples were weighed and processed using a microwave-assisted extraction method modified from "Microwave assisted acid digestion of sediments, sludges, soils, and oils" (EPA Method 3051A, without hydrofluoric acid, HF) for soils and EPA Method 3052 (without hydrofluoric acid) for plants. These methods have both been approved for processing samples containing Pb. Briefly, 0.2 g soil is mixed with 9 mL of trace metal grade nitric acid and 3 mL of trace metal grade hydrochloric acid, placed in a microwave oven for digestion and brought to a final volume of 50 mL with DI water (EPA, 2019). Plant samples were mixed with trace metal grade nitric acid ($HNO_3$) and 30% hydrogen peroxide ($H_2O_2$) in place of the HF acid to assist in digestion of organic material. The amount of sample needed for the method is determined based on the matrix and concentration of elements (EPA, 1996), but following the manufacturer's (Milestone Ethos UP Microwave Digestion System) directions, 0.2 g of dried, ground plant material was added to 6 mL of trace $HNO_3$ and 2 mL of trace $H_2O_2$ then brought to a final volume of 50 mL.

Each digest batch included two digestion vessels designated as blanks and two vessels containing NIST Montana I 2710a or NIST Montana II 2711a for quality control purposes. Blanks were treated the same as other vessels with the exception that no sample was added to them, just the reagents – this ensured that all reagents, filters, and glassware were free of contamination. Each digest batch also included a randomly chosen sample duplicate to ensure quality assurance and control. The digests were analyzed for Pb using ICP-OES following EPA SW-846 Method 6010D (EPA, 2018).

Soil samples from the four fields were characterized for pH, organic matter, texture, nitrate ($NO_3$), phosphorus (P), potassium (K), and calcium carbonate ($CaCO_3$) as well as total, water-soluble, exchangeable, and weak acid extractable Pb concentrations. To determine the pH, a saturated paste method was used (FAO, 2021). Electrical conductivity (EC) was also determined on the same saturated paste extract. Organic matter (OM) was characterized using the Walkley-Black method by oxidizing the organic matter with a known amount of chromate in the presence of sulfuric acid (Organic Matter - Walkley-Black Method, 2021). Nitrate and K were characterized on a 1:5 soil:water extract and P was characterized using the Olsen P method with 0.5 *N* sodium bicarbonate solution adjusted to pH 8.5 (Extractable Phosphorus - Olsen Method, 2021). The presence or absence of $CaCO_3$ was determined by applying a few drops of 10% hydrochloric acid on the soil. If the soil "fizzed" it was assumed to be to the result of a reaction between the acid and $CaCO_3$, releasing $CO_2$.


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

To evaluate the bioavailability of soil Pb to the corn, sequential extractions were done using a variety of reagents, each stronger than the last (Tessier et al., 1979). The first extraction was with DI water to determine the water-soluble fraction of Pb. For each sample, 2 g of soil was weighed into a 50 ml centrifuge tube with 10 mL DI water and shaken for 1.5 hrs. The samples were then placed in the refrigerator for 4-7 days to allow settling of soil. The supernatant was transferred to a 15 mL centrifuge tube for analysis of Pb on the ICP-OES and the remaining soil was used for the next extraction.

To the same 2 g of soil left from the first extraction, 16 mL of 0.1 *M* magnesium chloride was added and shaken for 1.5 hours to extract the cation exchangeable Pb ($Mg^{2+}$ exchanging with $Pb^{2+}$). The samples were placed in the refrigerator to allow the soil settle and then decanted in to 15 mL tubes for Pb analysis using ICP-OES.

The third extraction used the same 2 g of soil recovered from the first and second extractions. Sixteen mL of 0.005 *M* diethylenetriaminepentaacetic acid (DTPA) adjusted to pH 5 was added to the soil and shaken for 1.5 hrs. The samples were placed in the refrigerator to let the soil settle and then decanted into 15 mL tubes for Pb analysis using ICP-OES.

*Figure 3. Sequential extraction process in the lab conducted by Jaime Grijavla.*

5. Description of results; 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.

*Plant Samples:* All the corn husks and leaf samples from all four fields were below 0.007 ppm, the minimum detection level (MDL) for the ICP-OES used to analyze samples. However, corn roots had Pb concentrations ranging from 4.9 to 8.1 mg/kg (or ppm, Table 1). The standard error was calculated in Microsoft Excel using the equation:

$$\mathrm{SE} = \frac{\sigma}{\sqrt{n}}$$

Where the sample of the standard deviation (σ) is divided by the square root of the number of samples, n.

Results from all four fields indicate that the concentration of Pb in the corn leaves, husks, and kernels did not exceed Food and Agriculture Organization/World Health Organization recommended limits of 0.05 ppm (FAO/WHO, 2017). However, root Pb concentrations were above the limits and may be a concern if livestock is able to forage for corn roots. Even though we tried to thoroughly wash all soil from the root balls, some of soil (as high as 53 ppm Pb) may have adhered to the roots. Other authors have found that heavy metals such as

Pb may accumulate on, or in, the roots of various plants (Khan et al., 2015; Pourrut et al., 2011).

Table 1 The mean and standard error of Pb concentrations in the corn roots digested from each field (n=12).

| Field | Mean | Standard Error |
|---|---|---|
| | mg/kg | |
| Field 1 | 6.23 | 0.935 |
| Field 2 | 4.88 | 0.871 |
| Field 3 | 6.43 | 0.885 |
| Field 4 | 8.12 | 1.956 |

*Soil Samples:* The soil texture in the four fields was variable and ranged from a clay (fine-grained) to a sand (coarse-grained) but was predominantly in the loam category (Table 2). Such variability is not uncommon in alluvial soils formed from deposition along rivers. Soils from all four fields were neutral to slightly alkaline in pH and were nonsaline (EC <4 dS/m). Two of the fields were calcareous, containing free $CaCO_3$ that reacted with hydrochloric acid. The OM ranged from a mere 0.38% in the coarsest textured Field 2 to 3.15% in Field 1. The nitrate content was low (less than 3 ppm) as would be expected at the end of the growing season, however the P ranged from 3.2 to over 60 ppm (Table 2).

Table 2. Selected soil properties of each field.

| **Property** | **Unit** | **Field 1** | **Field 2** | **Field 3** | **Field 4** |
|---|---|---|---|---|---|
| Texture | - | Sandy clay loam*, Clay, Sandy loam | Sandy loam*, Loamy sand, sand | Sandy clay loam | Silty clay loam*, Silty clay, Clay |
| Fizz ($CaCO_3$) | Y/N | N | N | Y | Y |
| pH | - | 7.1 | 7.1 | 7.6 | 7.6 |
| EC | dS/m | 0.70 | 1.67 | 2.12 | 1.19 |
| Organic Matter | % | 3.15 | 0.38 | 2.86 | 2.01 |
| Nitrate | mg/kg | 2.90 | 0.94 | 1.04 | 1.88 |
| Potassium | mg/kg | - | - | 42.2 | - |
| Phosphorus | mg/kg | 19.6 | 15.1 | 61.4 | 3.2 |

**Predominant texture*

Sequential extraction using water, magnesium chloride, and weak organic DTPA acid showed that the bioavailability of Pb varies with the extracting compound (Table 3). Aside from "total" Pb, resulting from digestion in concentrated nitric and hydrochloric acid, the highest amount of *extractable* Pb resulted from DTPA, a weak acid that is used to mimic the natural acids released by roots that allow uptake of metal micronutrients such as iron, copper, and zinc. The DTPA-extractable Pb ranged from 24 to 40% of the total Pb and could explain occasional detectable Pb concentrations measured in corn kernels grown in the Animas

Watershed, although none of the aboveground plant parts contained Pb above the MDL in this study.

Table 3. Average soil Pb concentrations after sequential extraction.

| *Field:* | *Water-soluble[1] Pb* | *Exchangeable[2] Pb* | *Weak-acid[3] Extractable Pb* | *Total[4] Pb* |
|---|---|---|---|---|
| | | | *mg/kg* | |
| Field 1: | 0.725 | 0.007 | 19.66 | 50.05 |
| Field 2: | 0.530 | 0.056 | 5.26 | 14.54 |
| Field 3: | 0.140 | 0.020 | 21.15 | 53.21 |
| Field 4: | 0.0002 | 0.018 | 6.46 | 26.73 |

[1] *Deionized water extract analyzed on ICP-MS with MDL = 0.00001 ppm.*
[2] *0.1 M magnesium chloride extract.*
[3] *0.005 M diethylenetriaminepentaacetic acid (DTPA) extract.*
[4] *Concentrated nitric and hydrochloric acid digested soil Pb concentration. Note that 400 ppm (mg/kg) is allowable by the US EPA in residential soils and was used as the most conservative benchmark for soil Pb levels in the watershed.*

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

The evaluation of soil Pb concentrations and bioavailability in agricultural fields across the Animas and San Juan Watersheds benefits several communities living in the region. These communities include members of the Navajo Nation, local growers and consumers in and around the Farmington and Aztec area, and several government and private agencies working on rehabilitation efforts in the region. These findings can also be used by future student researchers and provide important data and baselines for follow-up studies and studies to replicate or verify these results. The results from this project may inform the city about metal concentrations in the corn that is being irrigated by the Animas watershed.

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 estimated and shown below of as of 04/20/2021. Due to delays with technical difficulties with lab machinery during Covid-19 some analysis are in progress to be billed.

| Proposed Expense Components | Account Used |
|---|---|
| Salary (Student Employee) | $3200.00 |
| Fringe Benefits/ Lab Chemical Supplies | $634.63 |
| Travel to collect samples | $501.46 |
| Laboratory analysis | $216.00 |
| **Total** | $5183.26 |

8. List presentations you have made related to the project.
    - Presented: "**Evaluating Soil Lead Bioavailability in Agricultural Fields across Animas Watershed" 65th Annual New Mexico Water Conference, online, 2020.**

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.

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

    - Michael Whiting (Graduate Mentor) Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces
    - Jaime Grijalva, Undergrad Student, Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces
    - Kaitlin Marry, Undergrad Student, Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces
    - Dr. Kevin Lombard, Associate Professor of Horticulture and Superintendent, New Mexico State University Agriculture Science Center at Farmington, Farmington
    - Dr. April Ulery, Professor, Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces
    - Barbara Hunter, Lab Coordinator, Soil Science Laboratory, Department of Plant and Environmental Sciences, New Mexico State University, Las Cruces
    - Brandon Francis, Education Resources Coordinator, New Mexico State University Agriculture Science Center at Farmington

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

    - E-News article: New Mexico Water eNews - (NMSU Student Studies Lead in Corn Harvested from Animas Watershed)

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 currently a senior with an expected date to finish my Bachelor of Science in Environmental Science in December of 2021. I aspire to earn my Master of Science in Environmental Engineering and assist with water management within the southwest region. Funding opportunities provided by the NM WRRI have assisted me in understanding how research works and its importance in my career. Being part of a research team has helped me understand how to analyze and process data and the effort that goes into meaningful research projects.

Final reports will be posted on the NM WRRI website.

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