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Phytoremediation of mining contaminated soil and groundwater by hemp (*Cannabis sativa* L.) and locally adapted native plants

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Phytoremediation of mining contaminated soil and groundwater by
hemp (*Cannabis sativa* L.) and locally adapted native plants

by
Nathan Carpenter

A non-thesis research paper submitted as part
of the requirements for the degree of

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ABSTRACT

Phytoremediation is an environmentally friendly and cost-effective method of using plants to remediate soils and groundwater by extracting and accumulating contaminants in their tissues. Using locally adapted native plants is preferable to non-native species due to native species being accustomed to the environmental conditions allowing for increased plant growth and fitness. It is typical to use hyperaccumulator plant species since they will grow in soils with very high concentrations of metals and metalloids and show efficient ability to recover (phytoextraction potential) and accumulate (bioconcentration factor) the contaminants in its tissues.

This study focuses on using thirteen locally adapted native plant species and two non-native species to remediate soil and a shallow alluvial aquifer contaminated by historic mining practices in Butte, MT. A controlled greenhouse experiment using soil and groundwater from the North Side Tailings in the Butte Priority Soils Operable Unit was performed to test what plant species can tolerate the metal(loid)s present. Each species phytoextraction potential (PE%) and bioconcentration factor (BCF) was compared to a known hyperaccumulator *Cannabis sativa*, which shows the best metal(loid) tolerance, PE%, and BCF. One native species, *Artemisia ludoviciana*, showed similar tolerance and ability to accumulate and recover Cu, Mn, and Zn to *Cannabis sativa*, but its total recovery was 1-19 times worse. Whereas comparing the other test species showed a significantly different and worse ability tolerate, accumulate, and recover the contaminants. Future research should be done to investigate the plants ability to accumulate and recover contaminants in its root system and compare the greenhouse experiment to a field study.

Since hemp fiber is widely used in industrial manufacturing, further research to understand how hemp fiber quality is impacted when used concurrently for remediation and industrial purposes is important.

Keywords: phytoextraction potential, bioconcentration factor, trace metals, greenhouse experiment, hyperaccumulator, Butte, Montana

INTRODUCTION

Historic mining practices in Butte, MT have left behind mine waste highly concentrated in arsenic (As), cadmium (Cd), copper (Cu), manganese (Mn), and zinc (Zn) (Tucci & Icopini, 2012). The Parrot Tailings, North Side Tailings, and Diggings East Tailings are the most notable; they have been left untouched since mining ceased and are a significant source of soil and groundwater contamination in the area (Tucci & Icopini, 2012). This project will focus on the Northside Tailings and Diggings East Tailings located around Upper Silver Bow Creek (USBC) in the Butte Priority Soils Operable Unit technical impracticability zone (BPSOU). Groundwater wells in this area have static water levels ranging between 1 and 3.5 meters below surface with high contaminant concentrations. Recreational parks are planned to remediate and restore the landscape in USBC. All groundwater saturated soil will be left in place, and groundwater monitoring and management is planned to keep surface water and Silver Bow Creek contaminant-free (EPA, 2006).

Phytoremediation is an environmentally friendly and cost-effective method of remediating contaminated soils and groundwater that typically implements the use of mycorrhizae. Mycorrhizal fungi form a symbiotic, mutualistic relationship with plants that help establish an ecosystem, improve plant diversity, and increase plant productivity (Quoreshi, 2008). Locally adapted native plant species and mycorrhizae are accustomed to the environmental conditions, and local mycorrhizae will improve plant growth and fitness (Crooks, 2002; Brooks et al., 2004; Charles & Dukes, 2008; Funk et al., 2008; Rúa et al., 2016). Native plants have shown an equal or superior growth and fitness when grown in a system with non-natives (Daehler, 2003).

Traditionally industrial hemp (*Cannabis sativa*) had multiple uses, most importantly it has been a source of fiber and hemp seed oil. Today it is becoming popular among farmers in several states of the US, including Montana, as a multi-purpose agricultural crop and an ideal alternative for organic farming. However, the plant's alternative uses stretch far beyond agriculture. Hemp is a hyperaccumulator meaning that it can grow in soil with very high concentrations of metals and metalloids and concentrates the contaminants in its tissues (Pal & Carpenter, 2020).

In this study, I investigated via a greenhouse experiment which native plant species can withstand the contaminant concentrations found in the soil and groundwater from the Diggings North area, and their respective metal recovery and accumulation potential, to understand which species could be efficient in remediation purposes. In addition, *Cannabis sativa* was used as a non-native test species. Further, the author compared the accuracy of pXRF vs. acid digestion followed by ICP-OES as a method to quantify contaminant concentrations in the plants.

METHODS

Soil and Groundwater Collection

Contaminated soil and groundwater for this project were collected in the spring of 2019 in the Butte Priority Soils Operable Unit along Upper Silver Bow Creek (Figure 1). The soil was homogenized, and analyzed for As, Cu, Fe, Mn, Pb, and Zn by acid digestions and analysis via inductively coupled optical emission spectrometry (ICP-OES) at the Montana Bureau of Mines and Geology Analytical Laboratory.

Groundwater was collected from well GS-32S with three 55-gallon drums with a Double Stage Geosquirt 12V DC Purge Pump. Water was stored at room temperature and purged

with nitrogen gas for 1.5 hours a week. Groundwater chemistry and plant growth were monitored weekly.

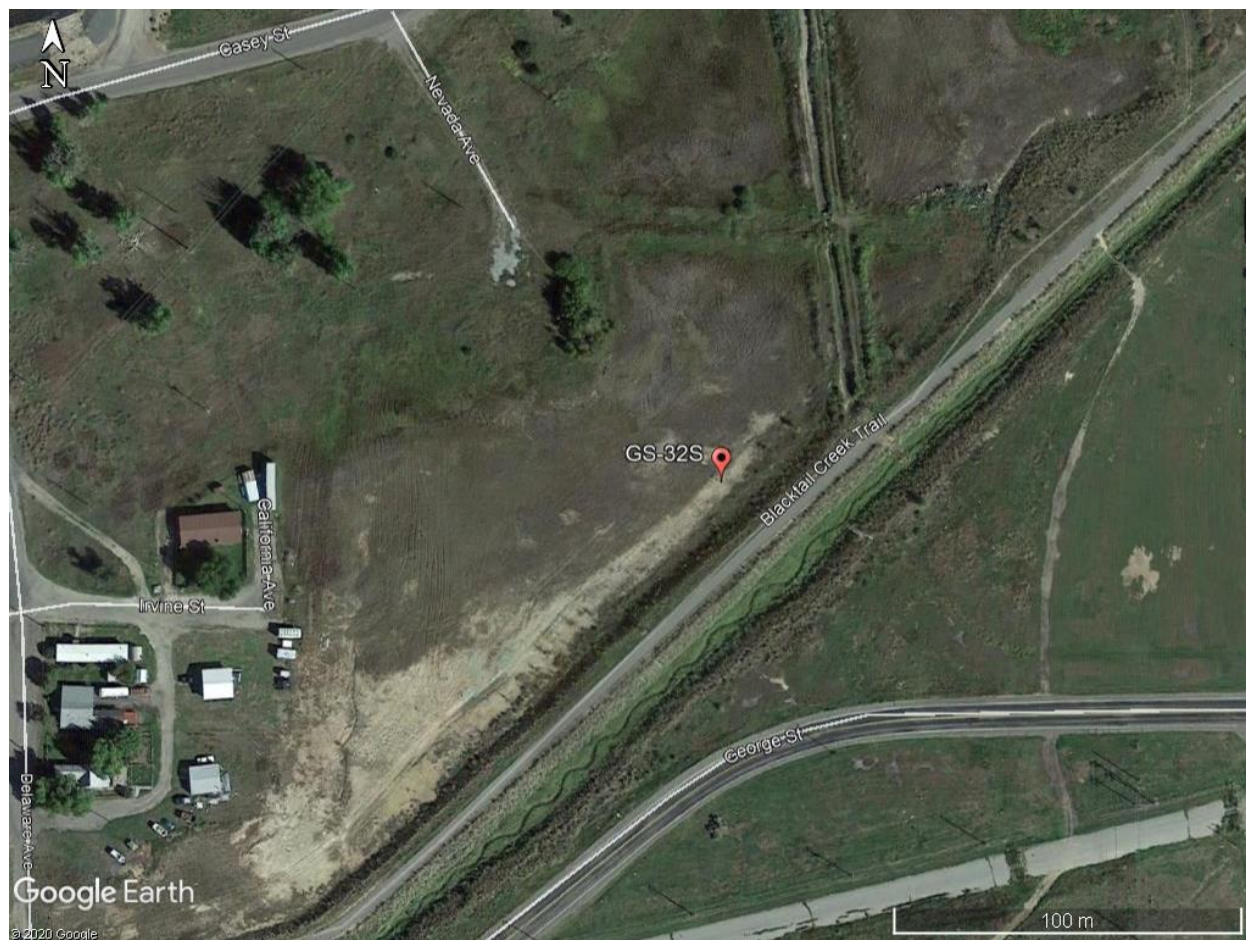


Figure 1. Location of soil and groundwater collection in the Butte Priority Soils Operable Unit. Soil was collected adjacent to the GS-32S groundwater well in Butte, MT.

Greenhouse Experiment

The greenhouse portion of this experiment was completed in two stages. The first stage included growing hemp (*Cannabis sativa*) and fourteen native plant species in contaminated soil. The native species were the following: balsam poplar (*Populus balsamifera*), quaking aspen (*Populus tremuloides*), sandbar willow (*Salix exigua*), bluebunch wheatgrass (*Pseudoroegneria spicata*), shrubby cinquefoil (*Dasiphora fruticosa*), white sagebrush (*Artemisia ludoviciana*), basin wildrye (*Leymus cinereus*),

rubber rabbitbrush (*Ericameria nauseosa*), big sagebrush (*Artemisia tridentata*), giant goldenrod (*Solidago gigantea*), common sunflower (*Helianthus annuus*), tufted hairgrass (*Deschampsia caespitosa*), Holbøll's rockcress (*Arabis holbolea*), and silky lupine (*Lupinus sericeus*). Seeds were germinated in a sterile petri dish with filter paper. Deepots D40L 656 mL growing pots were filled with soil collected from the study area, three cotton balls placed in the bottom, and half of the pots getting ½ inch inoculated layer of local mycorrhizae once the pot was ¾ full; mycorrhizae inoculum was collected from the West Side soils in Butte, MT (46°00'43.49" N, 112°34'14.40" W). Preparation of the pots consisted of washing with soap, a bleach soak, and then sterilization with 70% ethanol under a UV light.



Photo 1. Phytoremediation experiment. Photo credit: Robert Pal

The second stage included the same species as stage one, except for the balsam poplar and sandbar willow live cuttings, and shrubby cinquefoil were replaced with non-native wheat (*Triticum aestivum*), blanketflower (*Gaillardia aristata*), and hairy goldenaster (*Heterotheca villosa*). In this setting seeds were directly placed in the growing pots. Five

seeds were germinated in the pots and thinned down to one individual as germination occurred. Ray Leach Cone-tainers SC10 164 mL were used and filled with a soil mixture of 50% contaminated and 50% potting soil with a ¼ inch inoculated layer of local mycorrhizae once the pot was ¾ full. All plants were monitored weekly with growth measurements. One gram of Osmocote 19-6-12 fertilizer and inorganic phosphate were added to each pot. Inorganic phosphate will induce plants to accept the mycorrhizae since plants cannot uptake inorganic phosphate on their own. Initial watering for all plants was done by an overhead sprinkler system for five, three-minute cycles daily until plants were large enough for the implementation of groundwater (Photo 1).



Figure 2. Treatment groups for the greenhouse experiment.
No mycorrhizae indicate there will not be inoculation of mycorrhizae.

Each treatment group received weekly watering with either tap or contaminated groundwater, and either inoculation or no inoculation with mycorrhizae (Figure 2). Once watering with groundwater was implemented, all plants were in bins filled with either tap or groundwater, and they soaked for 1 hour once a week (Photo 2). Greenhouse temperature was kept at roughly 21°C during the day and at 18°C during the nighttime.

Plants were harvested after 16 weeks of growing. Plant samples were washed in tap water, roots and aboveground biomass length was measured. Afterwards plant samples were dried at 60°C for 48 hours and their above and belowground biomass was weighed separately.



Photo 2. Groundwater implementation to the greenhouse experiment.

Acid Digestion

For metals and metalloid analysis soil and plant matter samples were weighed out to a dry weight of 0.5 g and divided into roots and shoot/leaf matter. Samples were digested in 15 mL trace metal cleaned digestion vessels with 3 mL of Fisher chemical trace metal nitric acid at 120°C for 4 hours. Once vessels cooled, 1 mL of 30% J.T. Baker™ hydrogen peroxide was added at 70°C for 30 minutes. 15 mL of Q-water was added to each vessel and filtered through Whatman No. 40 0.2 µm paper into a trace metal clean secondary container. Digested samples were diluted to 30 mL with 1% nitric acid. ICP-OES data were corrected for dilutions to represent the mg of metal per kg of plant digested (e.g. converting Zn mg L⁻¹ to mg kg⁻¹) (Eq. 1):

$$mg\ Zn\ kg\ of\ plant^{-1} = \frac{1000 * Plant\ Zn\ OES\ concentration\ (mg\ L^{-1})}{mass\ of\ plant\ digested\ (g) * final\ digestion\ volume\ (L)} \quad (Eq.\ 1)$$

Groundwater Chemistry

Water samples collected for dissolved chemistry analysis were stored in 30 mL trace metal (TM) cleaned high density polyethylene (HDPE) Nalgene bottles. Samples were filtered across 25mm, 1.2 and 0.8/0.2 μ m polyethersulfone syringe filters using a 1 L HDPE Nalgene bottle, 140 mL polypropylene syringe, polycarbonate stop cock, and Tygon tubing all trace-metal cleaned. 30 mL bottles used for ICP-MS were pre-acidified with 300 μ L trace metal grade concentrated nitric acid (HNO₃), and ICP-OES samples were preacidified with 100 μ L extra pure, ACROS Organics™ methanesulfonic acid (MSA) Michalski et al., 2011; Oliveira et al., 2010). pH for each sample was measured with a WTW pH 3110 meter with an error of 0.01 and calibrated daily with pH 2, 4, and 7 buffers. Conductivity (μ S cm⁻¹) was measured with a YSI 30 meter with a conductivity error of 0.5%. Dissolved oxygen (DO) was measured with a PreSens Fibox 4 Trace meter that has a detection limit of 0.94 μ mol kg⁻¹ and an error of 0.4% (Robertson, 2019).

X-ray Fluorescence measurements

Four plant species and 10 replications were picked for method comparison analysis, which are: *Cannabis sativa*, *Artemisia ludoviciana*, *Triticum aestivum*, and *Pseudoroegneria spicata* (Photo 3). Species were chosen depending on how well they grew in the contaminated soil matrix, how they reacted to the introduction of groundwater, and how many replicates survived the greenhouse experiment. Pulverized soil samples and ground plant samples were placed into specialized sample cups designed for pXRF analysis, covered with a Mylar film, and labeled. Soil metals analyses were carried out with a

Thermo Scientific Niton XL3t GOLDD++ model analyzer in “Test-all Geo” mode. The prepared sample cups were placed into the pXRF test stand and tested by the remotely operated pXRF for 40 seconds each: 20 seconds in the Main Menu and 10 seconds for Light and Low elements. A background material tested was aluminum foil.

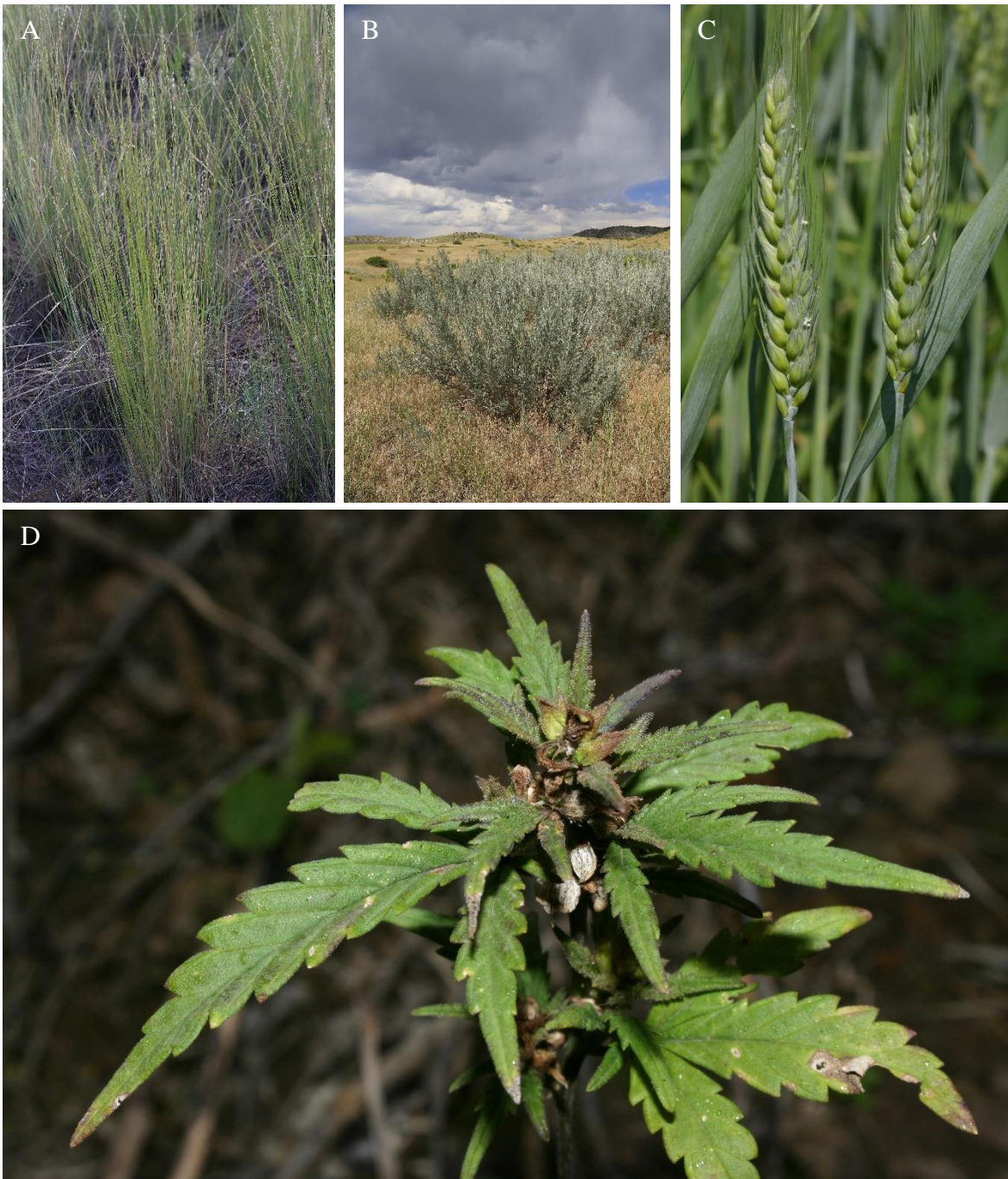


Photo 3. Plant species chosen for analysis via pXRF and ICP-OES. A: *Pseudoroegneria spicata*, B: *Artemisia ludoviciana*, C: *Triticum aestivum*, D: *Cannabis sativa*. Photo Credit: Robert Pal.

Method Comparison Analysis

To test the accuracy of the pXRF, the results were compared to ICP-OES results via the *Method Comparison Regression* (mcr) package in RStudio (Manuilova 2014). The Deming method was used because it quantifies the relationship between two measurement methods; it addresses regression errors in both variables (x- and y- axis) without repeated measurements unlike the simple linear regression model, where the explanatory variable is assumed to be measured without error.

To determine if the datasets are statistically similar, the US EPA standards were used to establish data quality. R^2 values greater than 0.85 meet the “definitive” quality level, and r^2 between 0.70 and 1.0 meets the “quantitative” quality level, and less than 0.70 meets the “qualitative” quality level (US EPA 1998).

Phytoextraction Efficiency and Bioconcentration Factor

Phytoextraction efficiency (PE%) (Eq. 2) looks at the total recovery of metals that plants extract from soil (Yang et al., 2017). The mass of soil in each pot was 217.5 g, which was calculated from the volume of the growing pots (164 mL) multiplied by the density of the soil; the calculated mass was divided by two since the soil matrix consisted of 50% potting soil. The density of the soil from the study area was determined by Tucci (2014), and the alluvial sand has a density of 2655 kg m⁻³.

$$PE (\%) = \frac{C_{metal\ concentration\ in\ plant} (mg\ kg^{-1}) \times W_{plant\ dry\ weight} (g)}{C_{metal\ concentration\ in\ soil} (mg\ kg^{-1}) \times (217.5\ g)} \times 100\% \quad (\text{Eq. 2})$$

It is typical to implement bioconcentration factors (BCF) (Eq. 3) when looking at the phytoextraction efficiency of species. BCF looks at the plants ability to accumulate from

the contaminated substrate (Wu et al., 2011). When plants have a bioconcentration factor of less than 1 it is assumed that this plant is not feasible for phytoextraction; however, it has been demonstrated that some species when grown on contaminated soils show great phytoextraction potential with bioconcentration factors less than 0.2 (McGrath & Zhao, 2003).

$$BCF = \frac{C_{concentration\ in\ plants}\ (mg\ kg^{-1})}{C_{concentration\ in\ soil}\ (mg\ kg^{-1})} \quad (\text{Eq. 3})$$

To compare PE% and BCF between species to understand “how many times greater” each species was to each other, the average of *Cannabis sativa* was divided by the average of each species. *Cannabis sativa* was chosen due to it being a hyperaccumulator and the only non-native test species.

Plant Species Metal(loid) Tolerance

A Kruskal-Wallis H test was used to understand how the tolerance to each contaminant compares between species. This is a rank-based nonparametric test that is used to determine if there is statistical difference between groups and does not assume the data variation is normal. The post-hoc Tukey Test was implemented on each species to determine if any species are statistically different (p-value <0.05). A result of ‘Do Not Test’ (DNT) occurs for a comparison when no significance difference is found between two means that enclose that comparison, and should be treated as no significant difference even if there appears to be one. For example, if you had four means sorted in order, and found no difference between means 4 vs. 2, then you would not test 4 vs. 3 and 3 vs. 2, but still test 4 vs. 1 and 3 vs. 1.

RESULTS AND DISCUSSION

Groundwater and Soil Chemistry

One groundwater well (GS-32S) was sampled for trace elements, major cations and anions;

In situ pH, conductivity, temperature, and dissolved oxygen were measured in the field.

165-gallons of the groundwater was stored and monitored weekly (Table 1A-D). Dissolved oxygen (DO) measurements were not collected *in situ* for 190924B and 190926B; the values were obtained from the “water isotope” sample vial. DO values increased from 5.77 to 7.09 mg L⁻¹ for the vial measurements. Weekly drum measurements show a decreasing DO value ranging from 5.52 to 0.68 mg L⁻¹ relative to a decreasing pH.

Table 1A. Groundwater parameters for sampling well and storage. D.O. = dissolved oxygen

Sample ID	Sample Name	T (°C)	pH	Cond. (uS/cm)	D.O. (mg/L)
190924B	GS-32S	21	2.432	2649	5.77
190926B	Drum 1 (GS-32S)	19.9	2.868	2664	7.09
191003B	Drum 1 (GS-32S)	20.2	2.736	2528	5.52
191010B	Drum 1 (GS-32S)	20.7	2.694	2465	2.47
191017B	Drum 1 (GS-32S)	19.7	2.665	2397	0.68

Major anions (Table 1B) increased in concentration during groundwater storage except for SO₄⁻² which decreased from 2777 to 2115 mg L⁻¹, and PO₄⁻³ decreased from 0.185 mg L⁻¹ to BDL two days after groundwater collection.

Table 1B. Major anions for the groundwater well and storage via IC. Units are in ppm; BDL = below detection limit.

Sample ID	Sample Name	F ⁻	Cl ⁻	Br ⁻	SO ₄ ⁻²	PO ₄ ⁻³	NO ₃ ⁻
190924B	GS-32S	4.85	108	BDL	2777	0.185	1.76
190926B	Drum 1 (GS-32S)	4.43	106	BDL	2696	BDL	1.92
191003B	Drum 1 (GS-32S)	5.54	113	0.088	2196	BDL	2.54
191010B	Drum 1 (GS-32S)	5.49	112	0.084	2198	BDL	2.52
191017B	Drum 1 (GS-32S)	5.35	112	0.086	2115	BDL	2.48

Major cations were relatively stable for the extent of groundwater storage except for: Ca^{+2} where the exhibited behavior shows depletion, K^+ shows slight enrichment within the first two days and depletion during storage, and both Na^+ and Mg^{+2} show depletion (Table 1C).

Table 1C. Major cations for the groundwater well and storage via IC. Units are in ppm; BDL = below detection limit.

Sample ID	Sample Name	Li ⁺	Na ⁺	K ⁺	Mg ⁺²	Ca ⁺²
190924B	GS-32S	0.324	61.4	5.66	88.4	396
190926B	Drum 1 (GS-32S)	0.317	61.4	6.41	85.6	380
191003B	Drum 1 (GS-32S)	0.322	58.7	6.38	85.1	377
191010B	Drum 1 (GS-32S)	0.326	58.1	6.20	84.8	376
191017B	Drum 1 (GS-32S)	0.326	58.1	6.34	85.7	379

Contaminants of concern were analyzed via ICP-OES since the concentrations were above the upper detection limit for ICP-MS; Arsenic is the only exception and was analyzed via ICP-MS. Throughout the groundwater storage, Mn and Cd did not show depletion or enrichment unlike the depletion of Fe, Cu, Zn, and As observed (Table 1D).

Table 1D. Contaminants of concern concentrations for the groundwater well and storage via ICP-OES in ppm. As was analyzed via ICP-MS and is in ppb. BDL = below detection limit.

Sample ID	Sample Name	Mn	Fe	Cu	Zn	As	Cd	Pb
190924A	Blank	BDL	BDL	BDL	BDL	BDL	BDL	BDL
190924B	GS-32S	20.8	122	120	100	793	0.475	BDL
190926B	Drum 1 (GS-32S)	19.4	102	110	92.6	304	0.442	0.196
191003B	Drum 1 (GS-32S)	20.5	81.4	110	80.0	105	0.424	0.210
191010B	Drum 1 (GS-32S)	20.4	74.1	111	80.0	66.3	0.415	BDL
191017B	Drum 1 (GS-32S)	20.5	73.1	112	80.8	58.6	0.422	0.192

Soil was collected from three distinctive layers (Photo 4) to be analyzed for bulk chemistry with pXRF and prepared for ICP-OES analysis via acid digestions (Table 2).

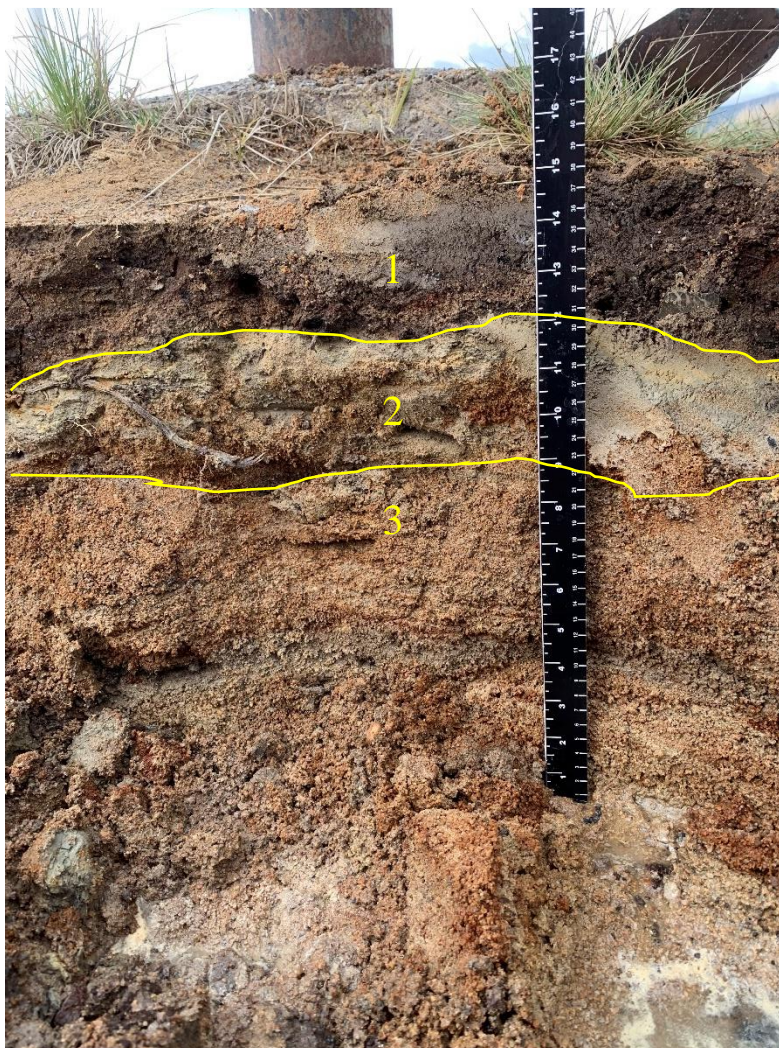


Photo 4. Wall of the soil pit showing the three layers collected for analysis. Yellow lines represent boundary lines between the organic, clay, and alluvial sandy loam.

Table 2. Soil digested mass and contaminants of concern concentrations analyzed via ICP-OES. Units are in ppm.

Sample Name	Mass (g)	As	Cu	Fe	Mn	Pb	Zn
Soil 1	0.5127	137	1281	22411	351	466	2417
Soil 2	0.5842	283	293	29065	196	364	2783
Soil 3	0.421	382	289	27292	167	324	1418

Changing Groundwater Chemistry

The lowest pH value seen in sample 190924B, which was measured at the time of sampling, is hypothesized to be a result of not purging enough volume of water out of the well. Groundwater concentrations for contaminants of concern did not change with storage except for Fe, Zn, and As which show depletion (Figure 3). pH is the dominating factor for arsenic examined in groundwater; at higher pH it is expected to have higher concentrations

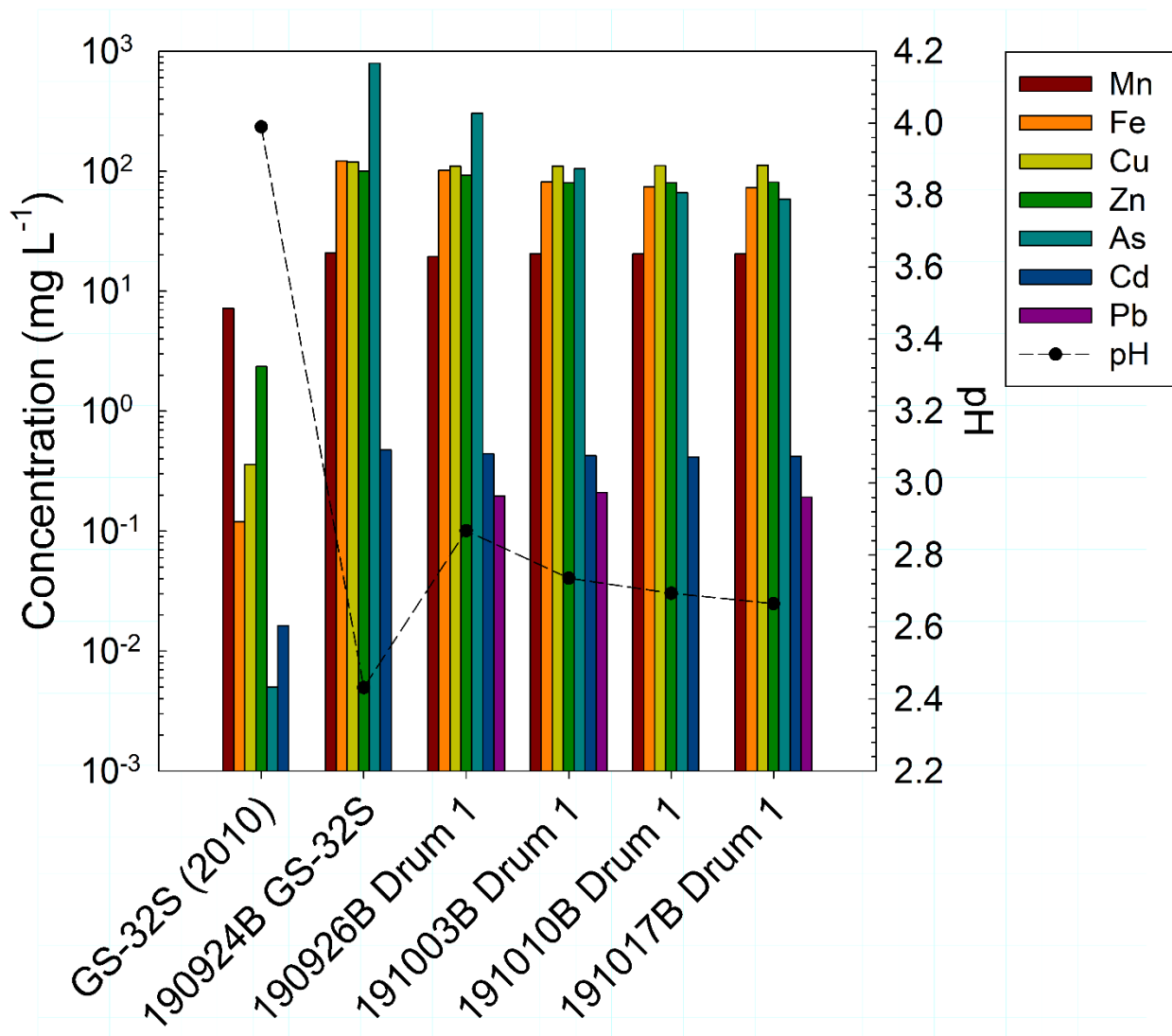


Figure 3. Changing groundwater concentrations. Note two y-axis; sample names year/month/day. Data for GS-32S (2010) was obtained from Montana's GWIC database. of As (Katsoyiannis & Katsoyiannis 2006).

Ferrous iron concentrations decreased from 0.12 mg L^{-1} to BDL from the precipitation of jarosite ($\text{KFe}^{+3}_3(\text{OH})_6(\text{SO}_4)_2$) or schwertmannite ($\text{Fe}_8\text{O}_8(\text{OH})_6(\text{SO}_4) \cdot n\text{H}_2\text{O}$). this precipitation was the likely source of the decrease in SO_4^{2-} concentrations observed (Figure 4). NH_4^+ and S^{-2} both have a significant increase two weeks after groundwater collection on 191010B. It is unlikely microbial activity induced the enrichment due to the D.O.

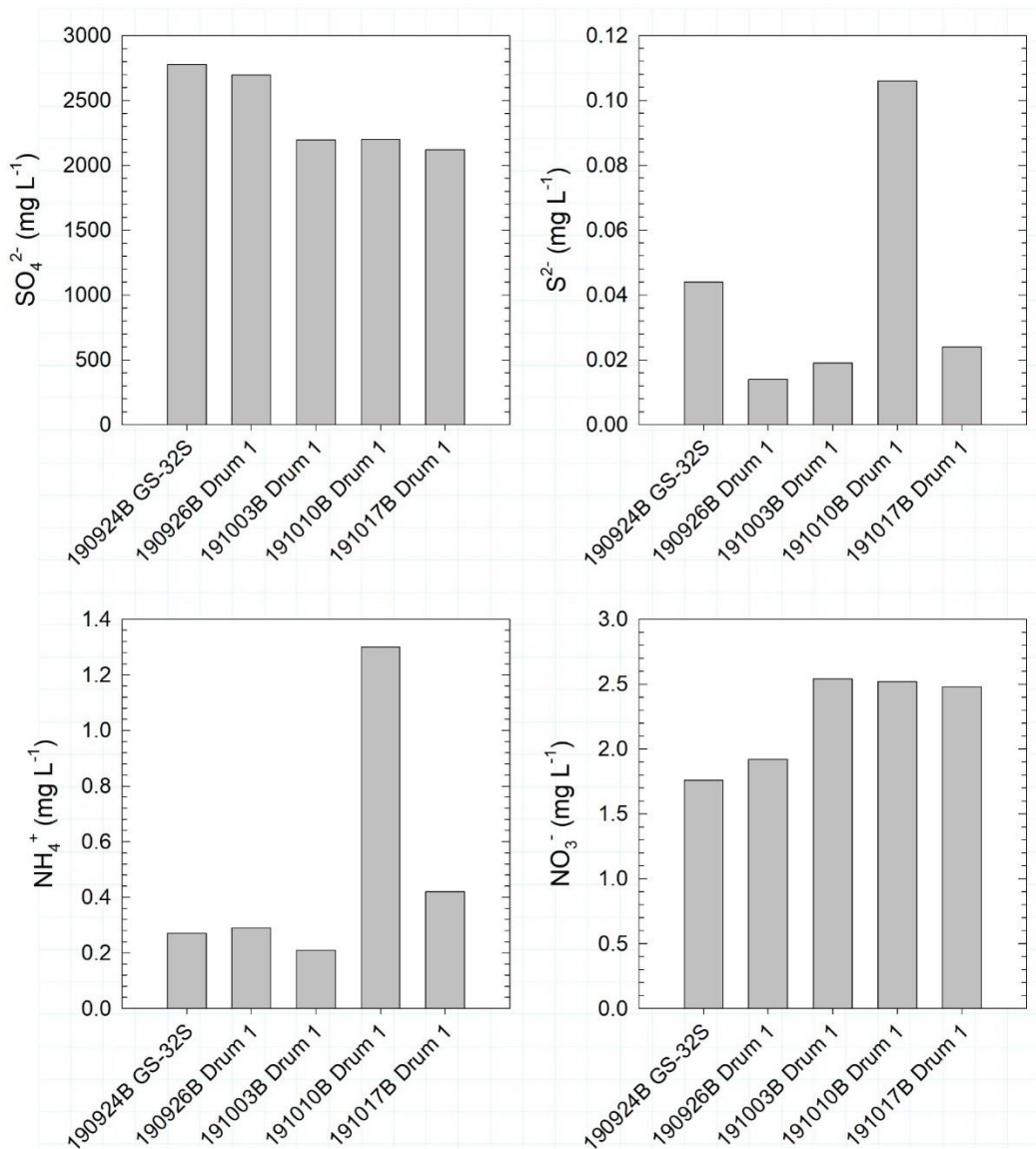


Figure 4. Field spectrophotometry for NH_4^+ and S^{-2} . SO_4^{2-} and NO_3^- concentrations measured via ICP-OES. Note that each y-axis has a different scale.

concentration being above 0.5 mg L^{-1} , and microbial samples for this week are stored at $-80 \text{ }^{\circ}\text{C}$.

Plant Growth

Weekly growth measurements were made for each plant species. All plant species show no difference between treatment groups during the growing phase. Once plants transitioned to the stationary phase, treatment groups with mycorrhizae are showing increased growth, where non-mycorrhizae groups show either a decrease or increase in growth (Figure 5). Once groundwater was implemented, *Cannabis sativa* and the grasses were either in the stationary phase (Figure 5A and D) or dying (Figure 5C). This is likely a result of the species being at the growing capacity for the growing pots. There was no evidence of the grasses dying or a negative impact on their health (i.e. discoloration, loss of chlorophyll, loss of biomass). *Cannabis sativa* was dying; there was loss of biomass and discoloration in the leaves. *Artemisia ludoviciana* was the only species used for analysis that was still in the growing phase after groundwater implementation (Figure 5B)

Plant growth rates were calculated by taking the natural log of each treatment group's average and determining the slope of the line, which is the growth rate (Figure 6A). The fastest growing plants species are the grasses which include wheat, basin wildrye, and bluebunch wheatgrass; one grass, tufted hairgrass, did not grow as fast. Mycorrhizae appears to have not influenced the growth rate of most of the plant species. The tree species, quaking aspen, and the sub-shrub (between a shrub and a wildflower, stems are woody) species, hairy goldenaster, both have the lowest growth rates besides giant goldenrod which did not germinate. The remaining forbs and shrubs have similar growth rates with variability coming from number of germinated seeds except for rubber rabbitbrush and Holbøll's rockcress, which are slow growers in the soil conditions.

Post groundwater implementation saw the opposite; plants that had a higher growth rate prior resulted in having a lower rate once groundwater was implemented (Figure 6b). Hemp is categorized as a forb, and compared to other forbs, the growth rate was at least double on contaminated soils.

Due to growth rates inhibited by the contamination present, most of the plant species did not have a long enough root system to reach the inoculated layer of mycorrhizal fungi, with the exception for the grass species and *Cannabis sativa*. Post-groundwater introduction (day 56) groups show an increased growth with mycorrhizae, with the groundwater treatment groups having the greatest increase. Two primary sources of this observation are: (1) the death of a replicate or (2) due to the high concentration of NH_4^+ and other nutrients in the groundwater. Plant species replicate deaths did occur, but the cause is most likely due to the heavy metal(loid) concentrations in the groundwater and soil. The abundance of nutrients from the groundwater most likely induced increased growth, whether the mycorrhizal fungi was accepted by the host plant. Even though *Artemisia ludoviciana* was still in the growing phase post-groundwater, the growth rate decreased from an average of $0.478 \text{ mm day}^{-1}$ to $0.143 \text{ mm day}^{-1}$ (Figure 6A-B), and was showing no signs of decreased plant health.

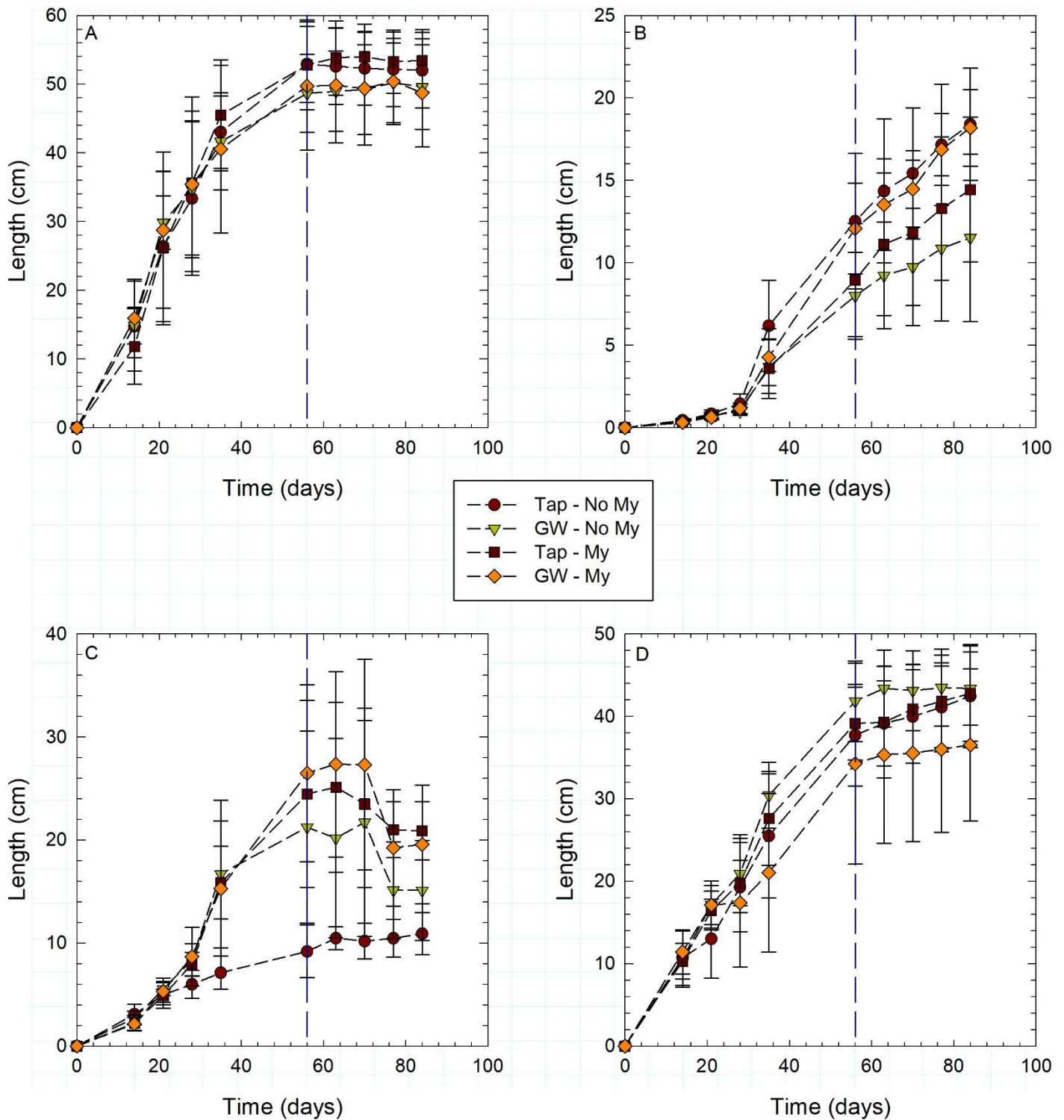


Figure 5. Plant growth during the greenhouse experiment. A: *Triticum aestivum*; B: *Artemisia ludoviciana*; C: *Cannabis sativa*; D: *Pseudoroegneria spicata*. Each data point represents the average plant length for each treatment group, with error bars representing the standard deviation. Vertical dashed line represents when groundwater was implemented.

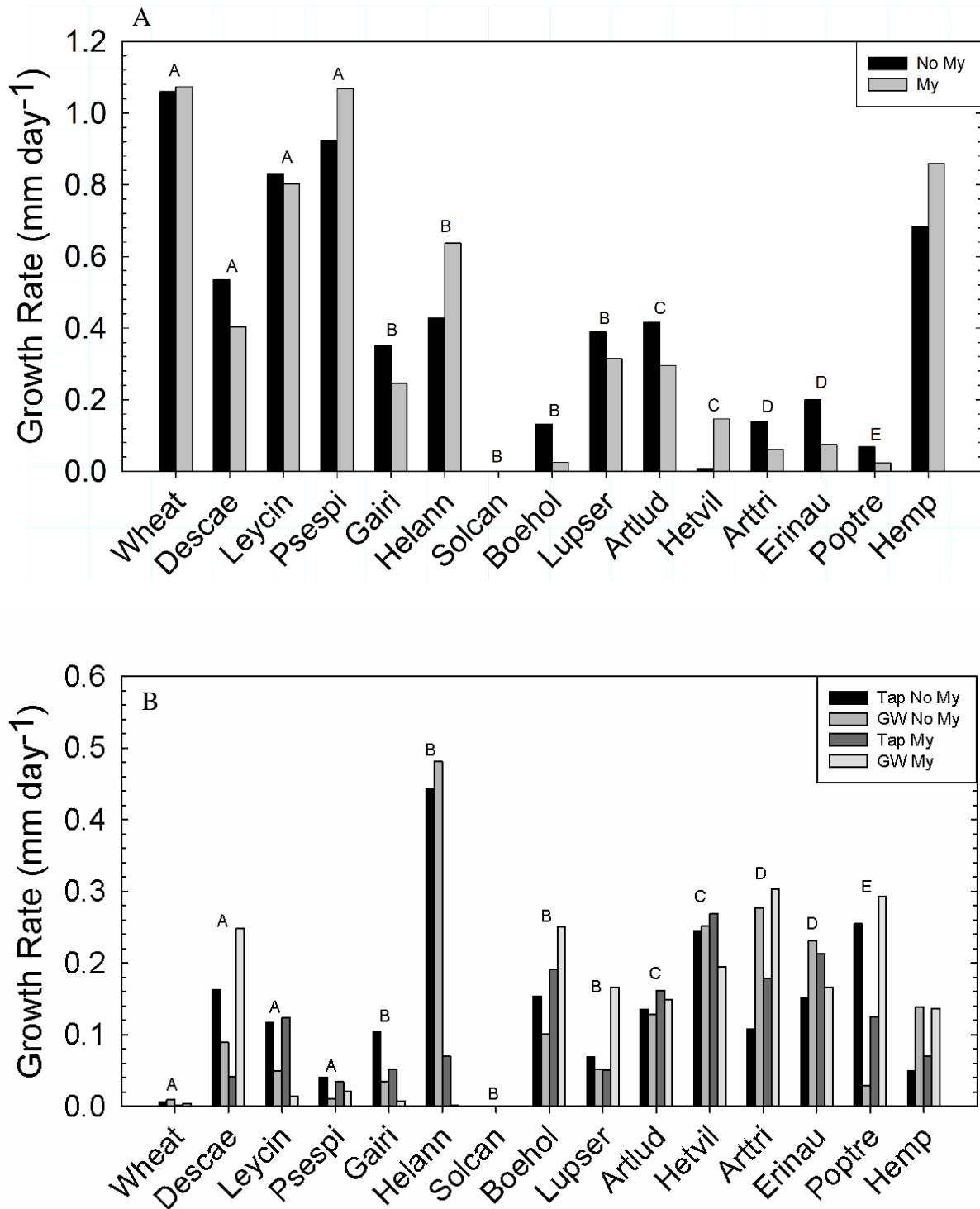


Figure 6. Plant growth rates. A: Pre-GW and B: Post-GW. Plant labels are the abbreviated scientific names of each species with the exception for wheat and hemp. Descae = tufted hairgrass; Gairi = blanketflower; Artlud = white sagebrush; Poptre = quaking aspen; Arttri = big sagebrush; Helann = common sunflower; Boehol = Holbøll's rockcress; Lupser = Pursh's silky lupine; Leycin = basin wildrye; Psespi = bluebunch wheatgrass; Hetvil = hairy goldenaster; Erinau = rubber rabbitbrush. A = Grasses; B = Forbs; C = Sub-shrub; D = shrub; E = tree.

Plant Species Tolerance to the Present Metal(loids)

To understand each species ability to withstand and adapt to the higher concentrations of toxic metal(loid)s found, a Kruskal-Wallis H test was conducted between each species and metal(loid) of concern. Among all plants, *Cannabis sativa* and *Artemisia ludoviciana* had the highest tolerance for all metals while *Triticum aestivum* showed moderate tolerance and *Pseudoroegneria spicata* had the lowest.

Copper

Cannabis sativa compared to *Pseudoroegneria spicata* and *Triticum aestivum* showed a significantly higher level of Cu tolerance at p-value (P)=0.016 and P =0.028, respectively (Table 3). Comparing *Cannabis sativa* to *Artemisia ludoviciana* and *Artemisia ludoviciana* to *Pseudoroegneria spicata* showed no significant difference at P =0.211 and P =0.643, respectively. ‘Do Not Test’ occurred for *Artemisia ludoviciana* to *Triticum aestivum* and *Triticum aestivum* to *Pseudoroegneria spicata*.

Table 3. Kruskal-Wallis H test results on Cu. DNT = Do Not Test

Comparison	Difference of Means	P	$P < 0.05$
Hemp vs Psespi	187.9	0.016	Yes
Hemp vs Wheat	174.0	0.028	Yes
Hemp vs Artlud	117.9	0.211	No
Artlud vs Psespi	70.0	0.643	No
Artlud vs Wheat	56.1	0.780	DNT
Wheat vs Psespi	13.9	0.995	DNT

For copper tolerance, *Cannabis sativa* showed the highest tolerance and *Artemisia ludoviciana* was moderately tolerant (Figure 7). *Artemisia ludoviciana* and *Triticum aestivum* overlapped at low concentrations and showed to be the least tolerable.

Manganese

As for Manganese levels in the test plants, *Artemisia ludoviciana* compared to *Triticum aestivum* and *Pseudoroegneria spicata* shows significant difference with $P=0.007$ and $P=0.010$, respectively. *Cannabis sativa* showed no significance between *Artemisia ludoviciana* and *Triticum aestivum* with $P=0.813$ and $P=0.063$, respectively. ‘Do Not Test’ occurred when comparing *Pseudoroegneria spicata* to *Cannabis sativa* and *Pseudoroegneria spicata* meaning there is no difference (Table 4).

Table 4. Kruskal-Wallis H test results on Mn. DNT = Do Not Test

Comparison	Difference of Means	P	P<0.05
Artlud vs. Wheat	296.30	0.007	Yes
Artlud vs. Psespi	286.5	0.010	Yes
Artlud vs. Hemp	75.400	0.813	No
Hemp vs. Wheat	220.90	0.063	No
Hemp vs. Psespi	211.10	0.081	DNT
Psespi vs. Wheat	9.80	1.000	DNT

For manganese tolerance, *Artemisia ludoviciana* was the most tolerable with *Cannabis sativa*; both overlap within their respective 95% confidence interval. *Triticum aestivum* and *Pseudoroegneria spicata* show the least tolerance and overlap at lower concentrations (Figure 7).

Iron, Lead, and Zinc

The differences in median values among plant species were not great enough to deduce whether the variation is due to random sampling variability; therefore, there is not a statistically significant difference between species for Fe, Pb, and Zn with a $P=0.079$, $P=0.176$, and $P=0.260$, respectively.

For iron, species concentrations overlap at all levels meaning that there is not a specific species that shows better tolerance (Figure 7). Fe and Zn are both a required micronutrient for growth and reproduction, so it is expected to see similar tolerance between species.

Lead was not detectable in all plant replicates from the ICP-OES.

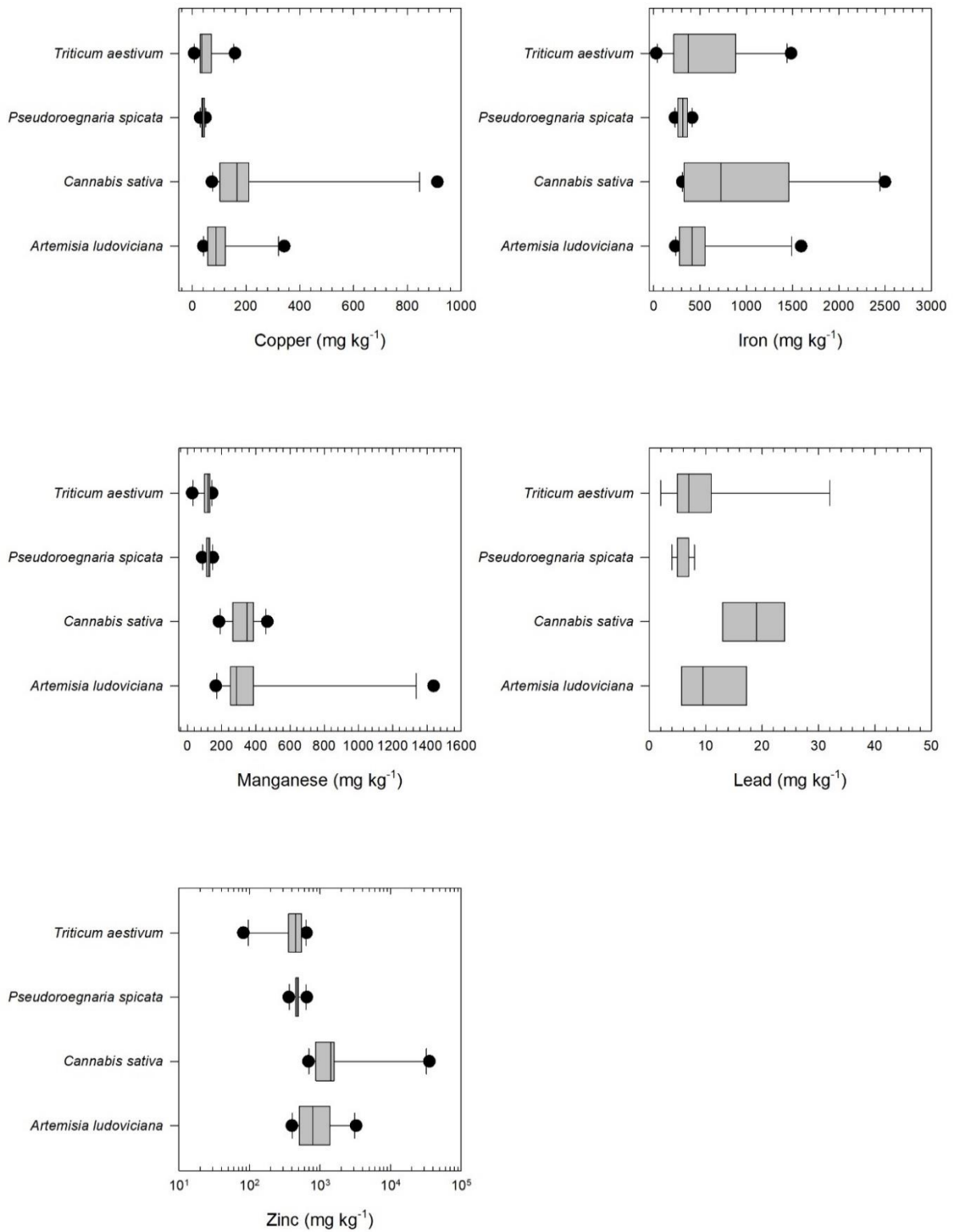


Figure 7. Plant species tolerance to metals of concern. The vertical line within the box represents the difference of means, circles indicate outliers, and error bars represent the 95% confidence interval. Arsenic was excluded due to being below detection limit from ICP-OES.

Phytoextraction Efficiency and Bioconcentration Factor

The average phytoextraction efficiency (PE%) and bioconcentration factor (BCF) of

Cannabis sativa was compared to each test species since *Cannabis sativa* showed the greatest metal(loid) tolerance (Table 5A-B); raw PE% and BCF values are summarized in Appendix A.

The average phytoextraction efficiency (PE%) of hemp was 3-8 times higher compared to the other test plants in the case of copper, 1-4 times higher for Fe, 1-2 times higher for Mn, 4-15 times higher for Pb, and 11-20 times higher in the case of Zn (Table 5A).

Table 5A. Comparing the average PE% of *Cannabis sativa* to each test plant.

Species	Cu	Fe	Mn	Pb	Zn
<i>Artemisia ludoviciana</i>	6	4	1	15	19
<i>Pseudoroegneria spicata</i>	8	4	2	11	20
<i>Triticum aestivum</i>	3	1	1	4	11

The average bioconcentration factor (BCF) of *Cannabis sativa* was 2-6 times higher compared to the other test species for Cu, 2-3 times higher for Fe, 1-3 times higher for Mn, 4-6 times higher for Pb, and 4-11 times higher in the case of Zn (Table 5B).

Table 5B. Comparing the average BCF of *Cannabis sativa* to each test plant.

Species	Cu	Fe	Mn	Pb	Zn
<i>Artemisia ludoviciana</i>	2	2	1	4	4
<i>Pseudoroegneria spicata</i>	6	3	3	6	10
<i>Triticum aestivum</i>	4	2	3	4	11

Plant tolerance of Cu differed significantly for *Cannabis sativa* when compared to *Pseudoroegneria spicata* and *Triticum aestivum*, but the PE% between species was 8 and 3 times higher, respectively. *Artemisia ludoviciana* and *Triticum aestivum* show no statistical difference between total recovery, and *Artemisia ludoviciana* shows no significant difference with its ability to accumulate compared to *Cannabis sativa*. *Artemisia ludoviciana* showed similar metal tolerance to *Cannabis sativa* while the PE% was 6 times lower. When looking at the ability to accumulate Cu from the contaminated soil, *Artemisia ludoviciana* and *Triticum aestivum* should, respectively, accumulate 2 and 4 times less than *Cannabis sativa*; however, *Triticum aestivum* exceeded expectations by recovering 3 times less than *Cannabis sativa*, and *Artemisia ludoviciana* underperformed and recovered 6 times less than *Cannabis sativa*. Even though *Cannabis sativa* and *Artemisia ludoviciana* both have the best Cu tolerance, *Triticum aestivum* shows the most similar recovery of Cu from the contaminated soil.

There is no significant difference between Mn and Zn tolerance comparing *Cannabis sativa* to *Artemisia ludoviciana*, *Pseudoroegneria spicata*, and *Triticum aestivum*. However, *Artemisia ludoviciana* is a sub-shrub and shows significant difference of metal tolerance between the two grasses *Pseudoroegneria spicata* and *Triticum aestivum*, but not *Cannabis sativa*, which is a forb. *Cannabis sativa* can accumulate 3 times more Mn than the grasses, and *Artemisia ludoviciana* is able to accumulate the same as *Cannabis sativa*. *Triticum aestivum* performed better than expected by recovering the same as *Cannabis sativa* and *Artemisia ludoviciana* when it should have recovered 3 times less. When looking at the ability to accumulate Zn, *Cannabis sativa* should accumulate 4 and 11 times more than *Artemisia ludoviciana* and *Triticum aestivum*, respectively. *Triticum aestivum* recovered

what was expected, but *Artemisia ludoviciana* underperformed by recovering 19 times less Zn than *Cannabis sativa*.

Artemisia ludoviciana did not transition to a stationary phase meaning that the species was still growing by the end of the greenhouse portion; this might have had an influence on the PE% and BCF. Therefore, if time in the greenhouse was extended for species still growing during groundwater implementation it would be expected to see *Artemisia ludoviciana* with similar or greater metal(loid) tolerance, PE%, and BCF than *Cannabis sativa*, which is a hyperaccumulator.

Comparing the Outcomes of pXRF and ICP-OES Analyses

The pXRF overreported all contaminants 1 to 10 times more than the ICP-OES analysis with the exception for a few samples close to 1:1 and 100:1 (Figure 8).

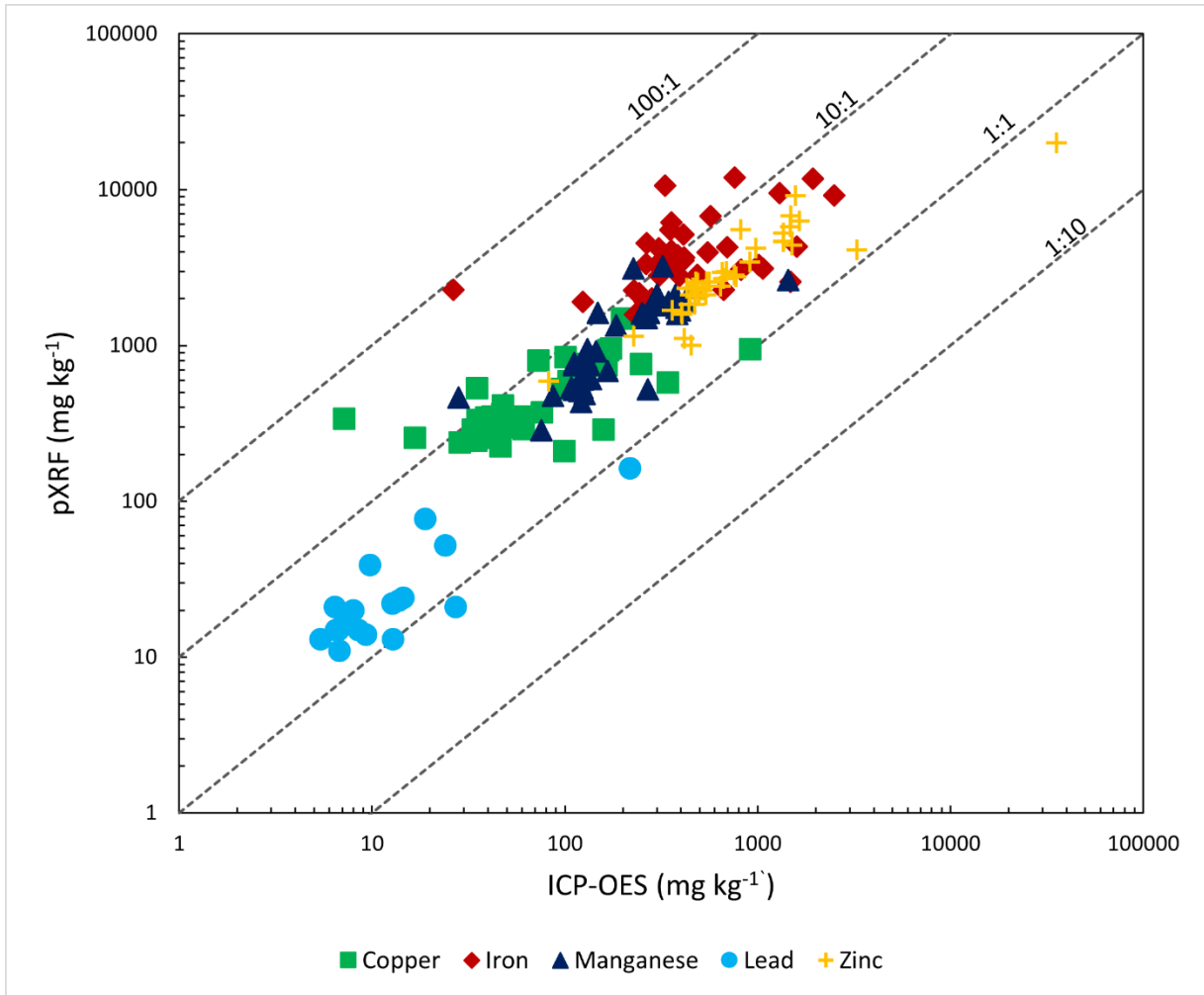


Figure 8. Method comparison of metal concentrations determined by pXRF and by ICP-OES for all plant samples. Dashed lines represent ratios between the two.

Looking at individual species (Figure 9A-D), *Pseudoroegneria spicata* shows the least variability in precision for concentrations unlike *Artemisia ludoviciana*, *Cannabis sativa*, and *Triticum aestivum*. There was no statistical difference found between treatment groups for each individual species.

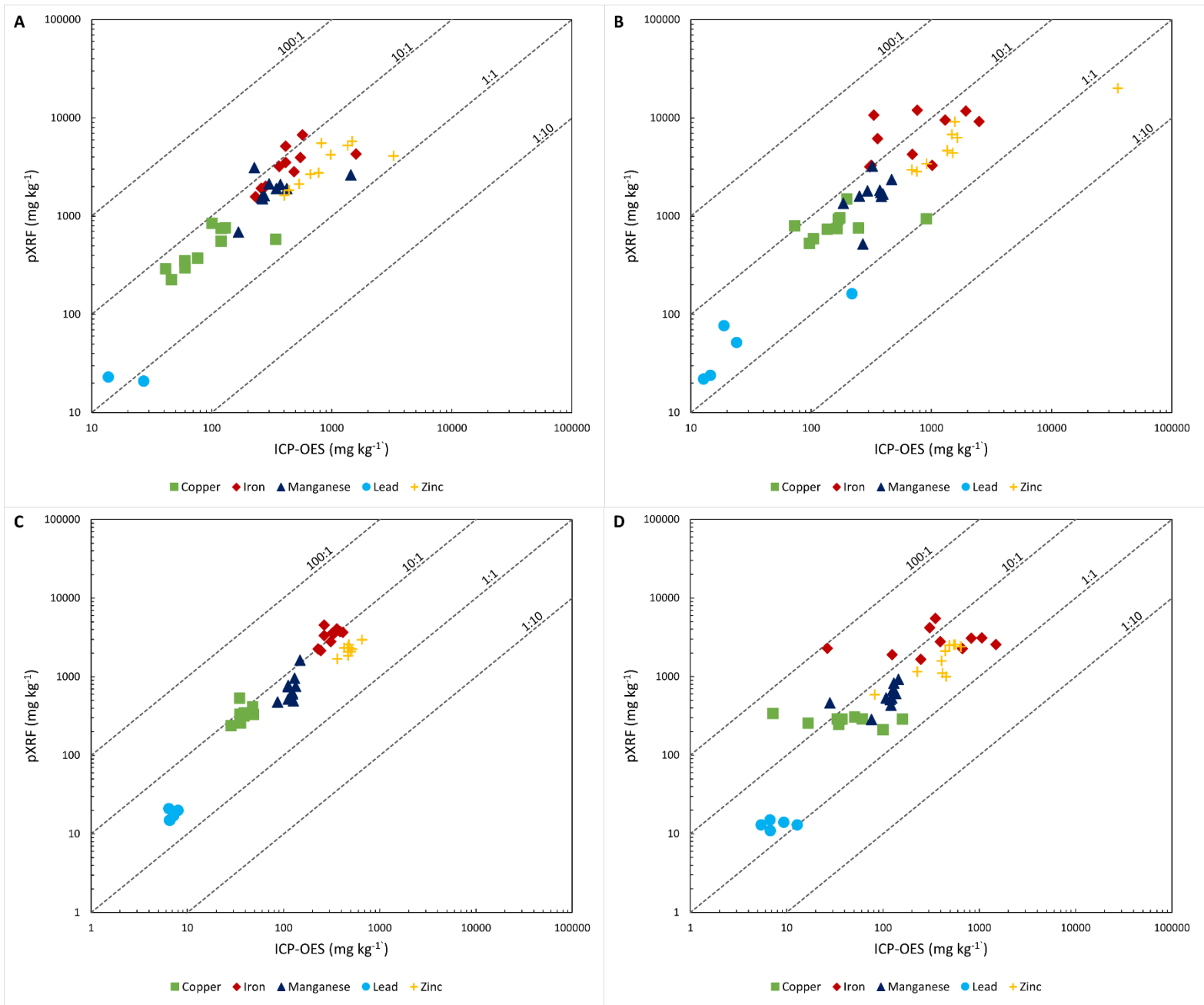


Figure 9. ICP-OES and pXRF concentrations for each plant species. Dashed lines represent ratios between the two. A: *Artemisia ludoviciana*; B: *Cannabis sativa*; C: *Pseudoroegneria spicata*; D: *Triticum aestivum*

To test the accuracy of the pXRF, the results were compared to ICP-OES results via a Deming regression in the *Method Comparison Regression* (mcr) package in RStudio, and the quality of data was determined by following the US EPA data quality guidelines (Manuilova 2014).

Zn and Pb had r^2 values of 0.868 and 0.917 respectively (Figure 10) which meet the US EPA standards of definitive data quality level, and agrees with the findings of Trilling (2018) besides Cu; however the number of observations for the Pb analysis was 19, and each other analysis had 40 with the exception of As. Arsenic, Cu, Mn, and Fe had r^2 values of 0.599, 0.521, 0.622, and 0.520, respectively, which is at the “qualitative” data quality level meaning that the datasets are statistically different.

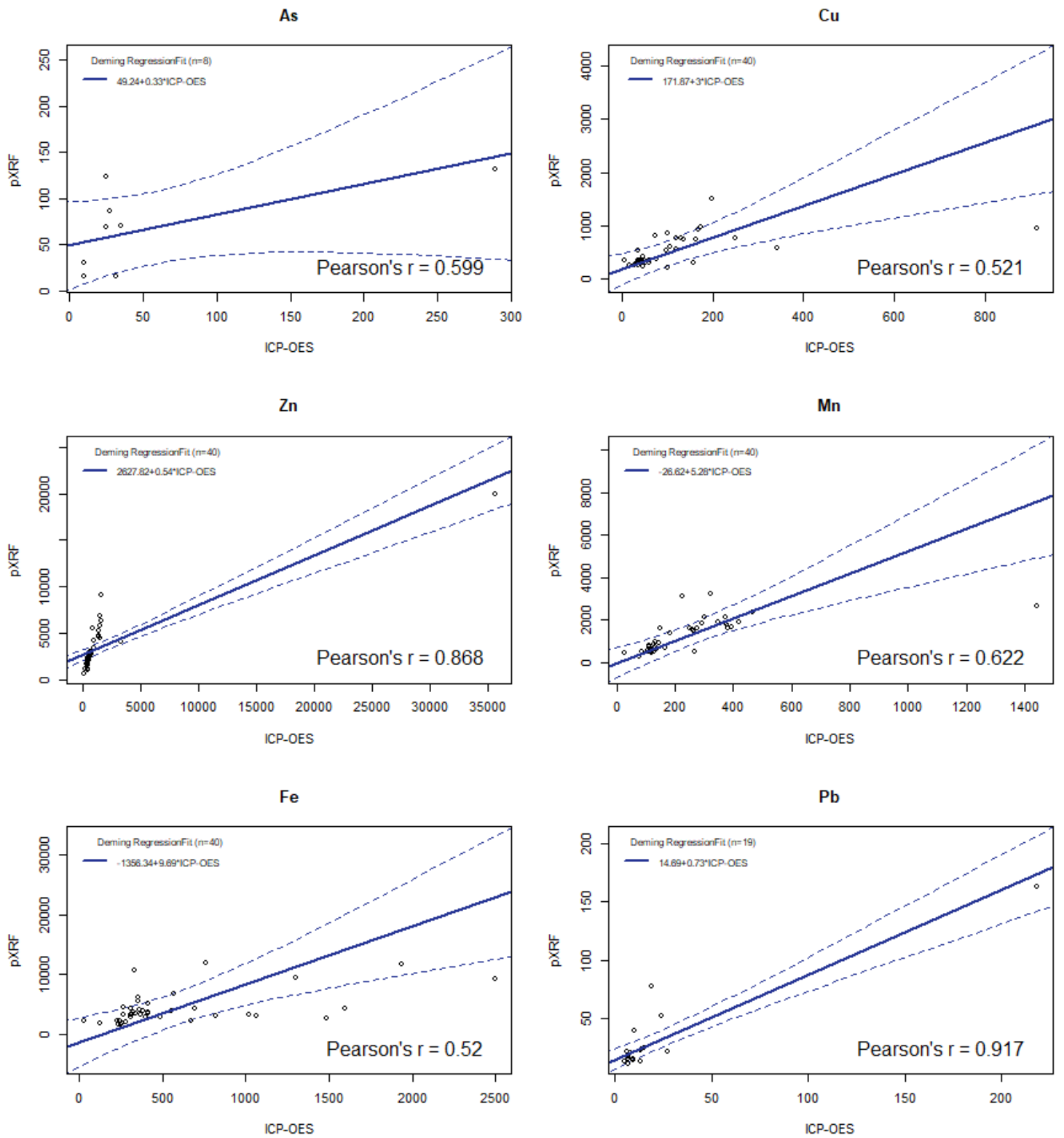


Figure 10. Deming regression analysis of pXRF against ICP-OES analysis. Deming relationship (solid blue line) and confidence intervals (dashed blue line) are shown on each plot; data is in units of mg kg^{-1} .

CONCLUSION

Decades of mining in Butte, MT has left a detrimental impact on the soil and groundwater quality within the community. We have demonstrated that plants will germinate and grow in a soil matrix of 50% contaminated soil and 50% potting soil but will have inhibited health and growth in 100% contaminated soil. The shallow alluvial aquifer will provide an abundance of needed nutrients to the plants. *Cannabis sativa* and *Artemisia ludoviciana* were the most tolerable to the heavy metals and metalloids present and both have great potential for use in remedial purposes. Future research on this topic should re-examine the greenhouse portion and implement the groundwater at the start or extend the length of groundwater treatment to provide the species with more time to extract and accumulate the contaminants, and also compare the results to a field experiment; comparing metal(loid) concentrations found in stems/leaves to the roots would help understand how the contamination is inhibiting the growth of the root system; a metagenomic survey of the soil, groundwater, and samples of the stored groundwater would allow us to understand what ecological interactions are happening on the microbial level. Further research on how the contamination impacted the quality of hemp fiber would be useful because it is important to understand how hemp fiber quality will be influenced when used concurrently for phytoremediation and industrial manufacturing purposes.

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APPENDIX

Table 3. pXRF data for replicates used in analysis; units are in ppm. BDL = below detection limit

Sample	As	Cu	Fe	Mn	Pb	Zn
Artlud 9	26	226	2012	689	BDL	1630
Artlud 12	51	844	5137	3123	26	5546
Artlud 13	29	581	4297	2624	21	4105
Artlud 14	32	555	2840	2100	BDL	5778
Artlud 15	44	751	6737	2130	39	5240
Artlud 17	19	763	3516	1622	21	4205
Artlud 25	37	351	3955	1504	23	1839
Artlud 28	26	291	3199	1550	15	2680
Artlud 37	17	373	1578	1913	BDL	2756
Artlud 39	24	297	1920	1906	BDL	2122
Hemp 2	51	799	10654	1586	43	2833
Hemp 3	54	528	3174	1681	BDL	3425
Hemp 10	69	587	11738	1838	24	2964
Hemp 16	83	929	6156	2361	58	4620
Hemp 18	86	958	3289	3222	31	4658
Hemp 28	132	944	9168	522	163	19966
Hemp 29	124	1493	11982	1349	77	9134
Hemp 34	87	744	9498	1749	52	4391
Hemp 36	165	759	3284	1811	BDL	6780
Hemp 39	70	738	4257	1603	22	6303
Psespi 1	25	240	3779	752	20	1676
Psespi 4	31	256	2807	520	BDL	1833
Psespi 14	19	349	3591	771	15	2089
Psespi 16	17	335	2154	488	BDL	2078
Psespi 31	28	317	2265	604	BDL	2327
Psespi 32	27	345	3535	476	15	2327
Psespi 33	23	332	3662	629	BDL	2940
Psespi 35	32	532	4544	1616	BDL	2260
Psespi 38	20	333	3329	948	21	2552
Psespi 39	23	411	4058	747	17	2347
Wheat 1	15	289	1664	510	BDL	1001
Wheat 3	19	256	1901	919	BDL	1105
Wheat 4	BDL	340	2282	465	BDL	591
Wheat 16	16	304	2788	607	BDL	2549
Wheat 17	19	291	3119	433	13	2118
Wheat 18	23	286	4194	537	13	2515
Wheat 19	24	288	5515	676	11	2551
Wheat 21	16	211	2280	536	14	1151
Wheat 23	16	246	3076	285	15	1588
Wheat 39	17	288	2562	825	BDL	2407

Table 4. Average pXRF for *Artemisia ludoviciana*; units are in ppm. NA = not measured

Sample	As	Cu	Fe	Mn	Pb	Zn
Artlud 1	14	94	599	258	16	404
Artlud 3	10	73	323	103	NA	187
Artlud 5	9	74	356	145	NA	365
Artlud 6	12	73	447	170	6	457
Artlud 7	10	232	522	158	11	450
Artlud 8	NA	44	712	NA	164	155
Artlud 9	26	226	2012	689	NA	1630
Artlud 12	51	844	5137	3123	26	5546
Artlud 13	29	581	4297	2624	21	4105
Artlud 14	32	555	2840	2100	NA	5778
Artlud 15	44	751	6737	2130	39	5240
Artlud 17	19	763	3516	1622	21	4205
Artlud 19	14	317	1831	1692	NA	2880
Artlud 20	23	712	3098	1112	NA	3532
Artlud 22	19	293	2517	452	NA	1220
Artlud 23	17	246	1841	634	NA	1510
Artlud 24	26	313	1970	641	NA	1283
Artlud 25	37	351	3955	1504	23	1839
Artlud 27	24	230	2147	615	12	1268
Artlud 28	26	291	3199	1550	15	2680
Artlud 29	19	260	2219	915	NA	1582
Artlud 32	21	346	1796	1362	NA	2824
Artlud 33	22	475	1977	1504	NA	2399
Artlud 34	14	270	1877	1041	NA	1640
Artlud 35	17	313	1781	772	NA	1684
Artlud 37	17	373	1578	1913	NA	2756
Artlud 39	24	297	1920	1906	NA	2122
Artlud 40	17	387	2455	962	NA	2569

Table 5. Average pXRF data for *Cannabis sativa*; units are in ppm. NA = not measured

Sample	As	Cu	Fe	Mn	Pb	Zn
Hemp 2	51	799	10654	1586	43	2833
Hemp 3	54	528	3174	1681	NA	3425
Hemp 6	66	369	2694	2455	NA	2344
Hemp 10	69	587	11738	1838	24	2964
Hemp 11	57	546	2577	467	NA	2719
Hemp 12	21	441	2978	1485	NA	1651
Hemp 13	43	702	3892	1467	18	3735
Hemp 14	36	309	2853	862	NA	2517
Hemp 16	83	929	6156	2361	58	4620
Hemp 17	65	719	6614	1273	59	3280
Hemp 18	86	958	3289	3222	31	4658
Hemp 21	47	565	11369	1021	26	2023
Hemp 23	47	343	3141	1119	29	1860
Hemp 25	65	741	4096	1114	39	4252
Hemp 27	22	309	2606	1284	NA	1804
Hemp 28	132	944	9168	522	163	19966
Hemp 29	124	1493	11982	1349	77	9134
Hemp 30	76	446	6845	1103	24	3125
Hemp 33	40	604	4258	1478	21	4853
Hemp 34	87	744	9498	1749	52	4391
Hemp 35	72	534	2963	1548	NA	3294
Hemp 36	165	759	3284	1811	NA	6780
Hemp 37	100	811	8946	1343	36	3225
Hemp 38	31	393	3308	1101	14	1971
Hemp 39	70	738	4257	1603	22	6303
Hemp 2	51	799	10654	1586	43	2833
Hemp 3	54	528	3174	1681	NA	3425
Hemp 6	66	369	2694	2455	NA	2344

Table 6. Average pXRF data for *Pseudoroegneria spicata*; units are in ppm. NA = not measured

Sample	As	Cu	Fe	Mn	Pb	Zn
Psespi 1	25	240	3779	3779	20	1676
Psespi 2	15	138	892	892	7	865
Psespi 3	15	162	1608	1608	NA	1398
Psespi 4	31	256	2807	2807	NA	1833
Psespi 6	18	219	2153	2153	NA	2624
Psespi 8	22	225	2614	2614	NA	1402
Psespi 10	18	183	2367	2367	NA	1287
Psespi 14	19	349	3591	3591	15	2089
Psespi 15	18	308	2814	2814	NA	1993
Psespi 16	17	335	2154	2154	NA	2078
Psespi 17	13	249	1618	1618	NA	1613
Psespi 18	14	311	2087	2087	NA	1998
Psespi 19	17	372	2552	2552	NA	1955
Psespi 20	18	347	2440	2440	NA	1788
Psespi 21	19	207	2192	2192	14	1603
Psespi 22	19	225	2256	2256	13	1206
Psespi 23	17	206	2031	2031	NA	1346
Psespi 24	30	255	2482	2482	NA	1838
Psespi 27	17	219	2606	2606	NA	1301
Psespi 28	20	219	2758	2758	12	952
Psespi 29	21	270	2537	2537	NA	1387
Psespi 31	28	317	2265	2265	NA	2327
Psespi 32	27	345	3535	3535	15	2327
Psespi 33	23	332	3662	3662	NA	2940
Psespi 35	32	532	4544	4544	NA	2260
Psespi 38	20	333	3329	3329	21	2552
Psespi 39	23	411	4058	4058	17	2347
Psespi 40	16	237	2025	2025	NA	1814

Table 7. Table 6. Average pXRF data for *Triticum aestivum*; units are in ppm. NA = not measured

Sample	As	Cu	Fe	Mn	Pb	Zn
Wheat 1	15	289	1664	510	NA	1001
Wheat 3	19	256	1901	919	NA	1105
Wheat 4	NA	340	2282	465	NA	591
Wheat 5	10	123	552	181	NA	690
Wheat 6	12	102	826	158	NA	999
Wheat 7	11	191	1295	568	NA	955
Wheat 8	12	172	1195	247	NA	1339
Wheat 11	11	217	1663	546	NA	1863
Wheat 12	14	252	2191	549	NA	1435
Wheat 15	13	241	1396	686	NA	2872
Wheat 16	16	304	2788	607	NA	2549
Wheat 17	19	291	3119	433	13	2118
Wheat 18	23	286	4194	537	13	2515
Wheat 19	24	288	5515	676	11	2551
Wheat 21	16	211	2280	536	14	1151
Wheat 23	16	246	3076	285	15	1588
Wheat 24	14	196	2251	188	15	1081
Wheat 25	13	173	1154	368	NA	1500
Wheat 26	16	217	1473	419	NA	1746
Wheat 29	13	180	1021	377		1038
Wheat 30	11	219	1469	320		1365
Wheat 31	9	189	985	227		929
Wheat 32	15	271	2014	458		893
Wheat 33	10	240	1754	227		1245
Wheat 34		134	1265			626
Wheat 37	21	241	3353	154	11	1251
Wheat 38	14	300	1980	159		1080
Wheat 39	17	288	2562	825		2407

Table 8. Corrected ICP-OES concentrations. Units in mg kg⁻¹. Blank cells = not measured

Sample	Mass (g)	As	Cu	Fe	Mn	Pb	Zn
Artlud 9	0.2578		46	282	166	6	401
Artlud 12	0.098		101	413	227		817
Artlud 13	0.0352		342	1594	1440	27	3264
Artlud 14	0.0611		120	485	374		1478
Artlud 15	0.0791		120	569	301	10	1362
Artlud 17	0.0322		130	412	274		978
Artlud 25	0.1552		60	549	265	14	439
Artlud 28	0.1432		41	365	260	9	664
Artlud 37	0.1788		76	232	421		773
Artlud 39	0.2622		60	259	346	5	535
Hemp 2	0.0657		73	332	383		758
Hemp 3	0.1695		97	308	396	5	913
Hemp 10	0.14	25	105	1939	373	15	688
Hemp 16	0.0165		168	356	467		1358
Hemp 18	0.0578		173	316	322		1360
Hemp 28	0.4802	289	912	2499	269	218	35610
Hemp 29	0.1654	25	198	762	185	19	1565
Hemp 34	0.201	28	164	1300	379	24	1507
Hemp 36	0.0519		249	1017	294	20	1486
Hemp 39	0.1392	35	136	694	252	13	1642
Psespi 1	0.1578		29	386	134	8	361
Psespi 4	0.3175	10	36	309	111	5	471
Psespi 14	0.1913		42	321	112	7	496
Psespi 16	0.2604		44	243	127	4	474
Psespi 31	0.1576		39	228	124	5	482
Psespi 32	0.1853		39	337	86	7	427
Psespi 33	0.2273		49	414	124	7	653
Psespi 35	0.0466		35	265	148		514
Psespi 38	0.1395		35	265	131	6	477
Psespi 39	0.1914		48	357	112	7	462
Wheat 1	0.3819		33	247	119	5	452
Wheat 3	0.488		17	124	145	2	416
Wheat 4	0.5109		7	26	28		82
Wheat 16	0.4621		51	394	136	7	562
Wheat 17	0.2874		61	1065	121	13	445
Wheat 18	0.409		35	306	123	5	488
Wheat 19	0.4835		37	351	126	7	546
Wheat 21	0.3734	10	100	668	108	9	227
Wheat 23	0.5071	32	35	822	76	7	405
Wheat 39	0.146		159	1484	129	32	643

Table 9. Uncorrected ICP-OES concentrations. Units are in mg L⁻¹. Bold numbers = below detection limit

Sample	As	Cu	Fe	Mn	Pb	Zn
Artlud 9	0.1	0.398	2.42	1.43	0.0487	3.45
Artlud 12	0.1	0.329	1.35	0.741	0.0237	2.67
Artlud 13	0.1	0.401	1.87	1.69	0.0318	3.83
Artlud 14	0.1	0.244	0.987	0.762	0.0237	3.01
Artlud 15	0.1	0.317	1.50	0.793	0.0257	3.59
Artlud 17	0.1	0.139	0.442	0.294	0.0237	1.05
Artlud 25	0.1	0.308	2.84	1.37	0.0711	2.27
Artlud 28	0.1	0.197	1.74	1.24	0.0406	3.17
Artlud 37	0.1	0.454	1.38	2.51	0.0237	4.61
Artlud 39	0.1	0.523	2.26	3.02	0.0447	4.68
Hemp 2	0.1	0.160	0.726	0.838	0.0237	1.66
Hemp 3	0.1	0.546	1.74	2.24	0.0305	5.16
Hemp 10	0.118	0.488	9.05	1.74	0.0679	3.21
Hemp 16	0.1	0.0922	0.196	0.257	0.0237	0.747
Hemp 18	0.1	0.334	0.609	0.621	0.0237	2.62
Hemp 28	4.63	14.6	40.0	4.30	3.49	570
Hemp 29	0.137	1.09	4.20	1.02	0.104	8.63
Hemp 34	0.186	1.10	8.71	2.54	0.161	10.1
Hemp 36	0.1	0.430	1.76	0.508	0.0346	2.57
Hemp 39	0.162	0.630	3.22	1.17	0.0590	7.62
Psespi 1	0.1	0.150	2.03	0.704	0.0421	1.90
Psespi 4	0.102	0.378	3.27	1.18	0.0578	4.99
Psespi 14	0.1	0.269	2.05	0.714	0.0415	3.16
Psespi 16	0.1	0.381	2.11	1.10	0.0359	4.11
Psespi 31	0.1	0.203	1.20	0.652	0.0280	2.53
Psespi 32	0.1	0.241	2.08	0.534	0.0407	2.64
Psespi 33	0.1	0.369	3.14	0.936	0.0520	4.95
Psespi 35	0.1	0.0542	0.412	0.230	0.0237	0.799
Psespi 38	0.1	0.164	1.23	0.607	0.0299	2.22
Psespi 39	0.1	0.304	2.28	0.715	0.0460	2.95
Wheat 1	0.1	0.426	3.14	1.51	0.0636	5.75
Wheat 3	0.1	0.271	2.02	2.36	0.0262	6.76
Wheat 4	0.1	0.122	0.450	0.477	0.0237	1.40
Wheat 16	0.1	0.778	6.07	2.10	0.108	8.65
Wheat 17	0.1	0.584	10.2	1.16	0.123	4.26
Wheat 18	0.1	0.481	4.17	1.68	0.0738	6.65
Wheat 19	0.1	0.602	5.66	2.03	0.109	8.80
Wheat 21	0.122	1.24	8.31	1.34	0.116	2.83
Wheat 23	0.540	0.586	13.9	1.28	0.113	6.84
Wheat 39	0.1	0.774	7.22	0.629	0.154	3.13

Table 10. Phytoextraction potential (%) calculated from ICP-OES data.

Sample	Cu	Fe	Mn	Pb	Zn
Artlud 9	0.019%	0.001%	0.101%	0.002%	0.017%
Artlud 12	0.016%	0.001%	0.052%	0.000%	0.013%
Artlud 13	0.019%	0.001%	0.119%	0.001%	0.019%
Artlud 14	0.011%	0.000%	0.054%	0.000%	0.015%
Artlud 15	0.015%	0.001%	0.056%	0.001%	0.018%
Artlud 17	0.007%	0.000%	0.021%	0.000%	0.005%
Artlud 25	0.015%	0.001%	0.096%	0.003%	0.011%
Artlud 28	0.009%	0.001%	0.087%	0.002%	0.016%
Artlud 37	0.021%	0.001%	0.176%	0.000%	0.023%
Artlud 39	0.025%	0.001%	0.212%	0.002%	0.023%
Hemp 2	0.008%	0.000%	0.059%	0.000%	0.008%
Hemp 3	0.026%	0.001%	0.158%	0.001%	0.026%
Hemp 10	0.023%	0.004%	0.122%	0.003%	0.016%
Hemp 16	0.004%	0.000%	0.018%	0.000%	0.004%
Hemp 18	0.016%	0.000%	0.044%	0.000%	0.013%
Hemp 28	0.688%	0.019%	0.302%	0.132%	2.825%
Hemp 29	0.051%	0.002%	0.072%	0.004%	0.043%
Hemp 34	0.052%	0.004%	0.179%	0.006%	0.050%
Hemp 36	0.020%	0.001%	0.036%	0.001%	0.013%
Hemp 39	0.030%	0.002%	0.082%	0.002%	0.038%
Psespi 1	0.007%	0.001%	0.050%	0.002%	0.009%
Psespi 4	0.018%	0.002%	0.083%	0.002%	0.025%
Psespi 14	0.013%	0.001%	0.050%	0.002%	0.016%
Psespi 16	0.018%	0.001%	0.077%	0.001%	0.020%
Psespi 31	0.010%	0.001%	0.046%	0.001%	0.013%
Psespi 32	0.011%	0.001%	0.038%	0.002%	0.013%
Psespi 33	0.017%	0.001%	0.066%	0.002%	0.025%
Psespi 35	0.003%	0.000%	0.016%	0.000%	0.004%
Psespi 38	0.008%	0.001%	0.043%	0.001%	0.011%
Psespi 39	0.014%	0.001%	0.050%	0.002%	0.015%
Wheat 1	0.020%	0.001%	0.106%	0.002%	0.028%
Wheat 3	0.013%	0.001%	0.166%	0.001%	0.034%
Wheat 4	0.006%	0.000%	0.034%	0.000%	0.007%
Wheat 16	0.037%	0.003%	0.148%	0.004%	0.043%
Wheat 17	0.028%	0.005%	0.082%	0.005%	0.021%
Wheat 18	0.023%	0.002%	0.118%	0.003%	0.033%
Wheat 19	0.028%	0.003%	0.143%	0.004%	0.044%
Wheat 21	0.058%	0.004%	0.094%	0.004%	0.014%
Wheat 23	0.028%	0.007%	0.090%	0.004%	0.034%
Wheat 39	0.036%	0.003%	0.044%	0.006%	0.016%

Table 11. Bioconcentration factor calculated from ICP-OES data. Blanks cells = not calculated

Sample	Cu	Fe	Mn	Pb	Zn
Artlud 9	0.158229	0.009689	0.848304	0.015565	0.144244
Artlud 12	0.344078	0.014218	1.156353		0.293662
Artlud 13	1.167585	0.054833	7.342471	0.074439	1.172785
Artlud 14	0.409294	0.016673	1.907269		0.530991
Artlud 15	0.410742	0.019573	1.533186	0.026771	0.489193
Artlud 17	0.442431	0.014168	1.396335		0.351476
Artlud 25	0.203398	0.018887	1.349979	0.037748	0.157651
Artlud 28	0.140997	0.012542	1.324271	0.023361	0.238604
Artlud 37	0.260241	0.007966	2.146863		0.277905
Artlud 39	0.204435	0.008897	1.761459	0.014047	0.192387
Hemp 2	0.249598	0.011406	1.950639		0.272336
Hemp 3	0.330149	0.010596	2.021047	0.014827	0.328127
Hemp 10	0.357255	0.066721	1.900726	0.039963	0.247138
Hemp 16	0.572709	0.012261	2.382031		0.487977
Hemp 18	0.59225	0.010875	1.643092		0.48858
Hemp 28	3.116143	0.085977	1.369445	0.598851	12.79425
Hemp 29	0.675426	0.02621	0.943111	0.05181	0.56239
Hemp 34	0.560897	0.044727	1.932573	0.066	0.541611
Hemp 36	0.849157	0.035002	1.496906	0.054932	0.533738
Hemp 39	0.463861	0.023876	1.285419	0.034924	0.590035
Psespi 1	0.097425	0.013278	0.682282	0.021983	0.12978
Psespi 4	0.122021	0.01063	0.568377	0.015	0.169402
Psespi 14	0.14412	0.011061	0.570797	0.017875	0.178047
Psespi 16	0.149958	0.008363	0.646026	0.01136	0.170123
Psespi 31	0.132016	0.007859	0.632688	0.014639	0.173032
Psespi 32	0.133299	0.011586	0.440721	0.018098	0.153564
Psespi 33	0.166385	0.014259	0.629759	0.01885	0.23473
Psespi 35	0.119207	0.009125	0.754814		0.184809
Psespi 38	0.120491	0.009101	0.665446	0.017661	0.17153
Psespi 39	0.162786	0.012295	0.571297	0.019803	0.166128
Wheat 1	0.114326	0.008486	0.60468	0.013722	0.162286
Wheat 3	0.056916	0.004272	0.739589	0.004424	0.14931
Wheat 4	0.024474	0.000909	0.142784		0.029536
Wheat 16	0.172556	0.013558	0.694995	0.019258	0.201763
Wheat 17	0.208263	0.036632	0.617262	0.035264	0.159766
Wheat 18	0.120534	0.010523	0.62818	0.014868	0.175251
Wheat 19	0.127611	0.012083	0.642093	0.018576	0.196177
Wheat 21	0.340357	0.022971	0.548818	0.025598	0.081691
Wheat 23	0.118438	0.028292	0.386024	0.018361	0.145387
Wheat 39	0.543344	0.051042	0.658864	0.086913	0.231075

Table 12. Weekly plant growth data for *Cannabis sativa*. Units are in cm. DEAD = when the replication died; blank cells mean no measurement.

Sample	Day 14	Day 21	Day 28	Day 35	Day 56	Day 63	Day 70	Day 77	Day 84
Hemp 1	3	5.6	6.5	7		DEAD			
Hemp 2	2.7	4.3	4.7	6.1	9.3	9.7	10.3	10.2	11
Hemp 3		4.1	5.7	9.6	12.1	12.2	12.6	12.6	12.7
Hemp 4	2.4	5.1	7.4	8.4	10.4	10.6	9.2	9.6	
Hemp 5	2.1	4.2	5.8	6.7					
Hemp 6	4.2	6.5	7.2	7.3	7.7	9.4	8	8	8
Hemp 7	3.5	5.4	5.6	5.5		DEAD			
Hemp 8	1.6	2.2	3.6	4.1	4.9	DEAD			
Hemp 9	4.1	6.6	8.1	8.3	DEAD	DEAD			
Hemp 10	4.2	5.7	5.4	8.2	10.7	10.4	10.8	11.9	11.9
Hemp 11	3	6.4	10.5	25.6	38.7	36.6	37.7		
Hemp 12	3.1	5.5	6.7	8	12	12.5	13.1	13.1	13.3
Hemp 13	2.4	5.5	7.2	11.3	15.1	15.4	16.3	16.2	16.2
Hemp 14	2.1	4.6	6.3	13.4	20.4	20.5	20.7	21.1	21.2
Hemp 15	2.9	5.4	6.8	7.5	9.1	9.1	10	10.1	9.7
Hemp 16	3.1	6.5	8.6	16.4	20.6	20.5			
Hemp 17	4.1	5.6	8.9	13.4	14.2	14.4			
Hemp 18	1.9	5.3	10.6	22.6	25.1	DEAD			
Hemp 19	2.6	5.8	9.8	21.5	24.7	DEAD			
Hemp 20	2.1	4.6	8.1	27.3	32.5	32.3	32.5		
Hemp 21	2	5	6.9	13	19.9	21	21.3	21.1	21.3
Hemp 22	1.8	5.5	9.2	16.5	22.6	22.8	23.1	22.8	22.9
Hemp 23	1.6	4.8	6.6	11	16	16.1	16.3	16.4	16.3
Hemp 24	3.7	6	9.1	16.25	20.3	21.2	21.1	21.8	21.2
Hemp 25	3.1	5.3	8	22.5	34.8	34.8			
Hemp 26	2.1	4.7	9.8	13.5	31	29.5			
Hemp 27	2.1	4.4	7.6	14.8	21.7	22	22.2	22.3	21.8
Hemp 28	2.3	4.6	7.7	15.7	23.1	23.4	23.4	24.6	24.6
Hemp 29		4.8	8.2	21	42.5	42.7	42.6		
Hemp 30	1.4	3.6	6.5	14.2	12.6	17.6	17.9	18	18.1
Hemp 31	2.5	6.5	8.6	18	22.5	DEAD			
Hemp 32	1.6	5	5.4	5.5	DEAD	DEAD			
Hemp 33	2.4	5.3	7.3	11.3	18.8	19	19.1	19	20
Hemp 34	1.9	4	5.5	8.2	13	13.6	13.7	13.7	13.6
Hemp 35	1.4	5.5	9.7	18.1	32.1	33	32.6		
Hemp 36	1.3	4	9.2	11.4	22.6	23.4			
Hemp 37		3.5	5.6	12.1	41	41.7	42.1		
Hemp 38	2.7	7.4	14.2	20.4	23.7	24.1	24.8	25	25.1
Hemp 39	3	6.8	11.5	26	32.5	32.2			
Hemp 40	2.6	5	9.8	21.9	32.1	31.8	31.5		

Table 13. Weekly plant growth data for *Artemisia ludoviciana*. Units are in cm. DEAD = when the replication died; blank cells mean no measurement.

Sample	Day 14	Day 21	Day 28	Day 35	Day 56	Day 63	Day 70	Day 77	Day 84
Artlud 1	0.3	0.6	0.8	3.5	8.3	9.8	11.8	14.5	16.4
Artlud 2	0.4	0.7	0.8	DEAD					
Artlud 3	0.4	1.1	1.5	5.9	10.6	12.1	13.2	15.5	16.7
Artlud 4	0.3	0.5	0.9	1.6	5.4	6.5	8	9.6	11.7
Artlud 5	0.6	0.9	2.5	8.9	16.8	18	18.9	19.5	21.8
Artlud 6	0.4	1.1	2.3	8.6	14.7	17.5	18.2	19.7	20
Artlud 7	0.5	0.6	1.1	3.6	10.3	12.8	13.6	15.6	16.2
Artlud 8	0.6	1.2	1.9	9.6	17.5	19.2	19.7	20.7	21
Artlud 9	0.5	0.7	1.4	6.9	15.6	18.3	18.6	20.7	22.1
Artlud 10	0.4	1	1.3	7	13.4	14.9	16.8	18.5	19.6
Artlud 11	0.2	0.5	0.6	1.6	3	3.1	3.6	4	4.3
Artlud 12	0.4	0.7	1.1	3.4	8.6	10	10.1	10.5	10.6
Artlud 13	0.4	0.8	1.2	4.2	9	11.5	13.5	16	18
Artlud 14	0.5	0.6	1.1	3.3	7.1	8.4	9.2	11.2	11.9
Artlud 15	0.3	0.6	1	5	8.7	8.8	9.1	9.5	9.8
Artlud 16	0.6	0.5	0.6	DEAD					
Artlud 17	0.4	0.8	0.9	3.7	7.6	9.1	9	9.6	10.2
Artlud 18	0.4	1.1	0.9	1	DEAD				
Artlud 19	0.3	0.7	1.2	5.7	12.5	14.6	15.4	18	19.6
Artlud 20	0.4	1	1.3	5.4	7.4	8.3	8	8.1	7.7
Artlud 21	0.4	0.5	0.7	2	8.7	7.5	8.1	9.3	10.7
Artlud 22	0.3	0.6	1	5.5	12	15.6	16.2	17.6	18.5
Artlud 23	0.4	1	1.5	5.7	12.1	15.4	16.3	17.5	18.6
Artlud 24	0.4	0.8	1.1	4.9	11.3	14	15.2	16.9	18.5
Artlud 25	0.3	1	1.2	3.1	10.2	10.7	11.2	12.7	13.3
Artlud 26	0.3	0.7	0.7	1.5	4	5.3	6.4	8.1	10
Artlud 27	0.2	0.7	1.5	4.6	9.9	12	12.5	14.1	15.3
Artlud 28	0.4	0.4	1.1	2	6.1	8.7	9.1	10.2	10.7
Artlud 29	0.4	0.8	1.3	5.3	12.2	16.8	17.6	19.4	20.4
Artlud 30	0.3	0.4	0.8	1.2	3	5.2	5.6	7	8.3
Artlud 31	0.3	0.4	0.7	1.5	6.7	8.7	10.4	13.2	14.1
Artlud 32	0.3	0.5	1.1	5.5	13.6	15.4	15.6	17.9	20.7
Artlud 33	0.2	0.5	1.5	6.1	15	16.4	16.7	18.9	17.9
Artlud 34	0.4	0.7	1.4	5.3	11.7	13.8	14.6	16.6	18
Artlud 35	0.2	0.5	1.1	6.4	15.6	17.7	18.3	20.8	22.4
Artlud 36	0.4	0.6	1	4.5	11.3	12.3	14.2	17.2	18.1
Artlud 37	0.3	1	0.7	2.6	8.9	10.8	12.2	14.7	16
Artlud 38	0.2	0.8	1.5	4	13.4	12.2	13.1	15.2	17.1
Artlud 39	0.5	0.8	1.6	2	13.6	15.6	16.1	17.8	19.4
Artlud 40	0.3	0.6	1.1	4.8	10.9	12.3	13.5	16.3	18.1

Table 14. Weekly plant growth data for *Triticum aestivum*. Units are in cm. DEAD = when the replication died; blank cells mean no measurement.

Sample	Day 14	Day 21	Day 28	Day 35	Day 56	Day 63	Day 70	Day 77	Day 84
Wheat 1		5	19.8	40.9	61.1	60.6	60.3	60.1	60
Wheat 2	11.3	25.7	37.3	44.1	60.1	60	58.7	59.1	59.1
Wheat 3	20.6	32.1	33.8	37.6	45.6	44.9	44.6	44.2	43.7
Wheat 4	9.1	27.7	38.3	47.6	47.4	47	46.8	46.5	46.7
Wheat 5	21.5	35.8	42.1	48.4	55.2	54.8	54.7	54.3	54.3
Wheat 6	17	36.5	42	45.4	51.5	51	51	50.5	50.4
Wheat 7	19.4	37.4	44.2	46.7	46.8	46.5	46.4	46.4	46.3
Wheat 8		15.2	34	47.3	55	54.8	54.6	54.5	54.3
Wheat 9	4.5	21.7	33.7	39.9	49.6	50.3	50.3	50.3	49.9
Wheat 10			8.2	32.2	56.5	55.9	55.6	55.5	55.4
Wheat 11			7.5	27.3	43	43.5	43.8	43.5	43.3
Wheat 12	18.4	35.8	41	47.4	55.1	54.8	54.4	54.5	51.2
Wheat 13	14.6	30.6	40.6	49.2	53.4	54.9	58.4	54.5	55.7
Wheat 14	19.7	35.2	40.5	41.1	48.2	47.4	47.5	47.5	57.4
Wheat 15	12.9	26.9	36.8	44	54.3	53.6	54.5	54.7	54.1
Wheat 16	14.8	26.9	35	35	44.6	43.6	44.4	44.3	44.2
Wheat 17	15.1	31.8	35.8	39.2	44.1	43.6	43.9	43.2	43.3
Wheat 18	13.9	29.7	32.7	37.5	46.2	45.8	45.6	45.9	45.8
Wheat 19	12.4	27.3	35.9	47.7	41.1	43.9	41	56.7	43.4
Wheat 20	11.6	24.6	41	48	56.4	58.5	58.4	57.1	57.3
Wheat 21	10.5	22.1	34.5	44.5	51.5	50.9	51.1	50.6	50.8
Wheat 22	9	22	29	41.8	47.6	49.3	49.3	46.2	48.8
Wheat 23	12.5	26.1	35.1	45.3	54	53.2	52.9	52.5	52.4
Wheat 24	17.9	40	44.8	52.5	56.3	55.6	55.5	55.2	54.8
Wheat 25	18.5	40.5	48.2	54.5	56	52.9	53.1	52.6	52.6
Wheat 26	10	15.6	25.7	37.8	55.7	60	59.6	58.3	58.6
Wheat 27	12.5	30.8	42.7	54.6	52.2	55.9	55.7	55.5	54.3
Wheat 28	0	4.5	14.2	29	37.1	42.3	44.8	44.8	44.9
Wheat 29	17.5	35.6	45.2	50.5	60.6	59.6	59.6	59.4	59.5
Wheat 30	9.5	24.6	36.6	44.2	56.8	58.4	58.3	57.7	57.2
Wheat 31	13.9	29.8	42.5	47.5	58.1	57.3	56.5	57.1	56.3
Wheat 32	13.2	26.3	38.9	49.5	55.1	55.4	54.6	55.1	54.9
Wheat 33	23.7	45.7	49	49.5	58.1	58.2	58.1	57.6	57.8
Wheat 34	19.6	40	45.8	45.3	49.7	49.2	48.7	48.2	48.4
Wheat 35	22.8	30	41.5	41	45	44.6	44.2	43.6	43.5
Wheat 36	15	35.9	42.7	46.2	50.1	49.6	48.2	49.6	49.4
Wheat 37	21.5	31.3	34.4	39.2	42.8	42.2	42.4	41.8	41.8
Wheat 38	12.9	27.7	32.3	45.2	55.9	56.3	56.1	55.8	50.2
Wheat 39	8	12.2	18.7	33.1	54.5	53.8	53.1	53.1	53.1
Wheat 40	8.5	8.6	8.6	9	27.8	31.6	31.6	42	31.8

Table 125. Weekly plant growth data for *Pseudoroegneria spicata*. Units are in cm. DEAD = when the replication died; blank cells mean no measurement.

Sample	Day 14	Day 21	Day 28	Day 35	Day 56	Day 63	Day 70	Day 77	Day 84
Psespi 1	12.3	14.6	18	23	30.1	34.3	34.1	35.5	35.5
Psespi 2	11.7	11.8	23.4	33.5	42.3	44.1	47.7	47.7	47.5
Psespi 3	10.1	17.8	23	26.5	43.1	43.3	43.2	42.8	47.6
Psespi 4		3.4	10.2	20.9	36.4	39.9	40.1	41.4	45.6
Psespi 5				9.3	29.1	32.2	34.1	41	41.8
Psespi 6	15.3	20.2	28.4	36.4	45.9	45.3	45.3	45.4	47.7
Psespi 7		11.9	19.1	26.1	42.5	42.5	45.4	47.8	49.5
Psespi 8		14.7	20.6	29.6	41.9	41.8	41.8	41.7	41.6
Psespi 9	5.2	10.2	13.9	22.1	31.3	31.1	31.1	31.1	31.1
Psespi 10	9.6	12.5	16.8	27.6	34.5	37	36.8	36.6	36.5
Psespi 11	11.9	17.1	22	32.7	43.6	47.9	47.6	47.7	47.1
Psespi 12	7	13.7	17.1	22	33.6	35.4	35.4	35.7	36.6
Psespi 13	9.1	16.7	23.5	30.2	47.1	46.8	46.5	46.4	46.3
Psespi 14	16.6	17.8	24.7	31.3	38.4	38.8	38.5	38.9	38.7
Psespi 15	7.2	17.2	24.9	32.6	43.5	43.5	43.5	43.4	43.3
Psespi 16	7.1	14	20.8	29.5	38.8	40.8	43.9	46.3	46.1
Psespi 17	7.6	13.5	19.2	26.3	38.8	44.3	44.1	44.1	43.9
Psespi 18	12.3	18.9	14.4	30.9	41.7	41.6	41.6	41.5	41.3
Psespi 19	14	22.1	28.4	36.9	51	51.7	51.6	51.2	51.1
Psespi 20	12.6	20.5	14.1	31.6	41.7	43	38.4	39.4	39.2
Psespi 21	12	16.3	16.2	24.5	37.5	40.6	40.4	39.7	43.4
Psespi 22	10.5	18.7	22.1	35.3	45	48.2	48.2	48.1	48
Psespi 23	8.5	14.5	17.8	24	33	32.9	32.8	32.4	32.6
Psespi 24	9	12.4	15.1	16	38	27.9	38.8	40	40.4
Psespi 25									
Psespi 26	11.5	16.2	21.7	28.4	34.9	37.2	36.8	37	37
Psespi 27	11.5	17.3	22.1	27	36.2	36.1	36.1	38.5	39.1
Psespi 28	8.7	15.9	20.1	32.9	45.4	48.5	48.6	48.4	48.3
Psespi 29	14	20.6	22.4	30.9	39.1	39.1	43.1	48.9	48.4
Psespi 30	7	16	20.5	29.4	43	43	42.9	43.3	47.6
Psespi 31	14	17.5	16.7	18.9	26.4	27.4	27.4	27.3	27.5
Psespi 32	6	14	16.6	22	40.8	40.1	40.4	40.2	40.1
Psespi 33	11	16.1	22.9	30.3	48.4	49.1	49.3	49.4	49.1
Psespi 34	13.8	20.1	24	28.6	40	39.9	39.8	39.8	39.7
Psespi 35			5	12.7	22	24.6	23.7	27.1	28.5
Psespi 36			4.2	12	22.7	24.8	27.2	28.3	30.5
Psespi 37				1.7	12.8	17.5	17.3	18.1	20.4
Psespi 38	11.2	14.6	20.4	25.2	39.7	40.9	40.9	40.7	40.6
Psespi 39	11.9	17.7	21.9	28.4	45.4	45	45.1	44.9	44.9
Psespi 40	12	19.8	24.7	30.5	44.2	43.9	44.4	44.1	44