Cadmium Uptake in Wheat: Effects of Phosphorus Management, Variety, and Nutrient
Uptake Dynamics;
Effects of Andic Mineralogy on Aluminum Toxicity in Acidifying Soils

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by

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Authorization to Submit Thesis

The thesis of Elizabeth Carp, submitted for the degree of Master of Science with a major in Soil & Land Resources and titled, " Cadmium Uptake in Wheat: Effects of Phosphorus Management, Variety, and Nutrient Uptake Dynamics; Effects of Andic Mineralogy on Aluminum Toxicity in Acidifying Soils," has been reviewed in final form. Permission, as indicated by the signatures and dates given below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Abstract

Cadmium accumulation in wheat threatens human health. Phosphorus fertilization management may influence cadmium uptake. Extensive research has investigated the effects of phosphorus application in cadmium-contaminated soils, but none has focused on Northern Idaho's natural soils. To investigate the effects of phosphorus application rate on cadmium accumulation, and on competition between cadmium and zinc for uptake, we conducted a greenhouse experiment applying varying phosphorus rates to spring wheat grown in natural soil. Additionally, relationships in grain and soil metal concentrations from across Idaho were analyzed using principal components analysis and regression modeling. Quintupling the recommended phosphorus rate resulted in a statistically insignificant increase in grain cadmium and did not induce antagonism between zinc and cadmium, indicating that phosphorus management may not be a viable strategy for controlling cadmium uptake in local soils.

Soil acidification can lower wheat yields by inducing aluminum toxicity, but little research has investigated the effect of acidification on Northern Idaho's ash-derived soils. Soils with and without andic properties at varying pH were analyzed for plant-available (KCl-extractable) aluminum to assess whether the effect of pH on aluminum toxicity changes in the presence of volcanic mineralogy. Soil pH was observed not to interact with andic status in its effect on Al availability. Additionally, plant-available Al was modeled on soil properties associated with ash. Soil pH, organic matter, and oxalate-extractable aluminum best explained exchangeable Al, while NaF pH, C/N ratio, and oxalate-extractable Al predicted organically bound Al.

Acknowledgements

Dedication

This thesis is dedicated to my brother Josh and sister-in-law Crystal, without whose support I could not have completed this project. I also give my heartfelt thanks to my graduate student cohort and my comrades in Palouse Democratic Socialists of America, whose friendship has filled my time in Moscow with joy.

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Chapter One: Controlling cadmium in wheat with P management

Introduction

Overview

Controlling cadmium (Cd) uptake in wheat is a public health priority. Cadmium is a trace metal that occurs in soils both naturally and through anthropogenic contamination such as industrial pollution and fertilizer application. It serves no biological purpose in any organism, and long-term consumption of food containing high concentrations causes health problems in humans, including kidney disease and bone demineralization. Wheat, which accumulates relatively high levels of Cd among cereal plants, is a potential source of Cd in the human diet (Grant et al., 2013). Understanding how environment and management impact Cd uptake in wheat is important to control its incidence in the food supply.

Two factors control Cd accumulation in wheat: 1) soil's ability to retain Cd, and 2) plants' ability to absorb and translocate Cd. The amount and speciation of Cd in soil influence plant accumulation. Mineral sorbents like manganese oxides may immobilize Cd, and organic matter can be up to thirty times as effective as mineral soil in sorbing trace metals (Baize et al., 2009; Sauvé et al., 2003). Soil pH, which affects mineral solubility and soil's capacity to adsorb Cd, may be the most important determinant of soil Cd availability (Lambert et al., 2007). DTPA-extractable Cd is considered to represent the Cd fraction that is mobile in soils and therefore available to plants (Baize et al., 2009). Biological characteristics like plant physiology influence Cd uptake and distribution throughout the plant, while microbial communities have been observed to both sequester trace metals in plant roots and encourage their uptake into above-ground tissue (Watts-Williams et al., 2013).

Competitive Absorption Between Zn and Cd

Zinc (Zn), which is similar in size and charge to Cd, is widely observed to compete with Cd. The mechanisms that control competitive absorption are unclear, but may involve competition for uptake via shared transport proteins, oxidative stress, and wheat's response to Zn deficiency. Hart et al. (2002) found reciprocal uptake inhibition between Zn and Cd, and suggested that the metals share a common transport system at the root-cell membrane. Zinc is

also involved in enzymatic processes that protect wheat from oxidative stress, which can damage cell membranes and make them more permeable to Cd. Gao et al. (2011) describe a decrease in one such enzyme, Cu/Zn superoxide dismutase, under Zn deficiency, along with damage to the root cell plasma membrane, which may allow nonselective uptake of Cd. Wheat may respond to Zn deficiency by up-regulating Zn-transporting proteins, or releasing root exudates that lower rhizosphere pH or chelate metals, increasing the availability of Cd as well as Zn (Chaney et al., 2006; Jiao et al., 2004).

Not all research demonstrates Zn-Cd antagonism. Some studies found no relationship, or even synergism, between the two (Grant and Bailey, 1998; Zhu et al., 2003). Xue and Harrison (1991) observed Cd-Zn synergism, reporting that in high-Cd soils, Cd uptake in lettuce increased at higher Zn application rates. They suggest that synergism may occur in high-Cd soils, while antagonism occurs at lower Cd concentrations. One proposed mechanism for synergism is that Zn can compete with Cd for complexation with phytochelatins, which enable plants to tolerate toxic metals by sequestering them in root cell vacuoles. Increased phytochelatin-Zn complexation could leave more Cd available for translocation to the grain (Sarwar et al., 2010). Excessive Zn concentrations may also decrease antioxidant enzyme activity, increasing reactive oxygen species concentrations and causing membrane damage that permits the nonselective uptake of Cd (Saifullah et al., 2014).

The most consistent difference between studies that found Zn-Cd antagonism and those that did not is the concentration of Zn in the soils. In Zn-deficient soils, applied Zn tends to reduce Cd uptake in wheat (Brennan and Bolland, 2014; Köleli et al., 2004). In soils with sufficient or excessive Zn, applied Zn is less effective or encourages Cd uptake (Grant and Bailey, 1998; Nan et al. , 2002; Zhu et al., 2003). Zinc deficiency may make cell membranes more permeable to Cd or induce a plant response that enhances Cd availability in the rhizosphere. Where Zn is deficient, added Zn is expected to decrease Cd uptake, while effects are more variable at adequate and excessive Zn levels.

Effects of P on Zn and Cd Bioavailability in Soils

Growers can influence the availability of Cd and Zn through management. For example, phosphorus (P) fertilizer application may influence both soil- and plant-based determinants of Cd uptake in wheat. When P is applied to soil, it can enhance Cd immobilization. In more weathered, variable charge soils, HPO_4^{2-} can adsorb to soil minerals

and make their surface charge more negative, enabling mineral surfaces to sorb Cd more strongly (Bolan et al., 2014). Applied P can also co-adsorb with Cd onto soil minerals and form complexes on phosphate compound surfaces (Rehman et al., 2015). Siebers et al. (2013) found the proportion of Cd sequestered in root cell walls increased with P addition relative to that in other cellular parts, and suggested this may be due to adsorption onto phosphate anions on the root cell wall. P can reduce Cd accumulation through effects on plant physiology like enhanced growth, which dilutes Cd. It can also encourage the synthesis of phytochelatins, which sequester Cd within the plant roots and prevent transport to the grain (Sarwar et al., 2010).

Many of the studies that found P reduced Cd uptake did not reflect typical wheat growing conditions: soils were contaminated with Cd or amended with very high P levels. P-induced Cd precipitation only occurs in highly contaminated soils (Arshad et al., 2016; Rehman et al., 2015), where Cd concentrations are close to equilibrium with respect to solubility of Cd-solid phases (Bolan et al., 2014). Indeed, many studies in which P fertilizer decreased Cd uptake took place on contaminated soils. Grant and Sheppard (2008) argue that P only reduces Cd availability at application rates higher than typical agronomic practice. Siebers et al. (2013) observed P-induced Cd suppression only occurred at P application rates far above normal usage. Wang et al. (2017) observed that in Cd-spiked soils labile Cd increased, and fixed Cd decreased at P application rates of 200 mg/kg. As P increased beyond this rate, labile Cd decreased and fixed Cd increased. This research suggests that absent severe industrial contamination and atypical P application rates, direct P-induced Cd suppression may be unlikely.

On the other hand, P fertilizer may also be associated with increased Cd uptake due to fertilizer contamination with Cd, soil acidification, increased ionic strength, increased plant growth, or decreased Zn availability. The Cd content of phosphate rock ranges from trace amounts to over 300 mg/kg (Grant and Sheppard, 2008). Phosphorus sources contain different levels of Cd contamination: Roberts (2014) reports that due to differences in the manufacturing process, the range of Cd concentrations in monoammonium phosphate is lower than in triple superphosphate. Different P fertilizers also have varying indirect effects on Cd availability: fertilizers that contain ammonium, such as monoammonium phosphate, can acidify soil due to nitrification, making Cd more available (Jiao et al., 2004). If Cd input

through contaminated fertilizer is greater than what is removed through crop biomass, Cd can accumulate in the soil, although this does not necessarily increase Cd accumulation in grain (Grant et al., 2013, Wang et al., 2014).

Phosphorous fertilizers have varying effects on pH, the soil parameter most strongly correlated to Cd uptake. They can acidify soil by producing phosphoric acid, as well as through nitrification of P sources that contain ammonium, increasing Cd availability. While P-enhanced plant growth is generally thought to reduce Cd concentration through dilution, Grant and Bailey (1998) argue that it may also encourage Cd accumulation. They found grain Cd concentrations correlated with yield, which they suggest may be a result of enhanced mass flow related to increased growth.

Phosphorus fertilization has been observed to reduce Zn availability, which, given the relationship between Zn and Cd, may impact Cd uptake. Many studies have documented the inhibitory effects of P fertilizer on Zn uptake (Grant and Bailey, 1998; Bogdanovic et al., 1991; Ova et al., 2015). On the other hand, some studies observed synergism. Zhao et al. (2005) found antagonism between Zn and P at low levels of applied Zn and synergism at higher Zn levels. Proposed mechanisms for Zn-P antagonism include immobilization of Zn by P in the soil, interference of P with Zn translocation, and metabolic issues related to P-Zn balance (Gao et al., 2011).

Recent research suggests P decreases Zn uptake through its influence on mycorrhizal colonization. Arbuscular mycorrhizae (AM) are widely observed to enhance plant Zn uptake. Phosphorus fertilizer tends to inhibit colonization, resulting in decreased Zn uptake. Ova et al. (2015) argue that this is the predominant mechanism for P-induced Zn suppression. They found adding P to an AM-colonized soil resulted in a greater decrease in Zn uptake than in an autoclaved soil, suggesting that inhibition of colonization accounted for the bulk of Zn depression in P-fertilized wheat. They also found P fertilization had no effect on DTPA-extractable Zn, suggesting that P's effects on soil chemistry did not account for Zn suppression. However, Austruy et al. (2014) found that P caused Zn immobilization only in the presence of plant roots. They suggested root exudates solubilized apatite, freeing P to form complexes with Zn. AM fungi have also been observed to limit trace metal uptake in plants. When trace metal concentrations are excessive, AM fungi can ameliorate toxicity to both themselves and host plants by preventing metal entry into the roots by chelating and

sequestering metals within their cells (Ferrol et al., 2016). Chen et al. (2003) found root metal concentrations in AM-infected red clover were significantly higher, and shoot concentrations lower, than their uninfected counterparts. They suggested this is likely due to metal retention on or in fungal mycelium, which can sequester ten times as much metal as host tissue grown in the same soil. Phosphorus may increase Cd uptake through its inhibition of mycorrhizal colonization, and therefore inhibit Zn availability. These studies show that there is not yet a clear understanding of the role of microbial community in metal uptake, and that further investigation is necessary.

Zn suppression could be the mechanism by which P has been observed to increase Cd in wheat. However, interactions between the three cannot simply be inferred from studying each in isolation. As with Zn, P could suppress Cd uptake by adsorbing it in the soil and enhancing the ability of roots and fungi to bind it to cell walls. AM fungi could increase Cd uptake, meaning P-induced AM suppression could in fact decrease Cd accumulation. The hypothesis that P increases Cd through Zn suppression may only hold if these mechanisms selectively inhibit Zn uptake over Cd—if Zn has a greater affinity for P-complexation or AM selectively absorb Zn.

Current Research Gaps

Many studies have examined interactions between P and Cd or Zn, but few have addressed interactions between the three. Those that did yielded varying results. Grant et al. (2010) and Jiao et al. (2004) found that added P decreased Zn and increased Cd in wheat grain; Bogdanovic et al. (1991) found P decreased Zn, but did not increase Cd; and Zhao et al. (2005) found Cd uptake increased with P rates and decreased with Zn rates, but P and Zn were actually synergistic, suggesting that P increased Cd through other means. The mechanisms of interactions between P, Zn, and Cd are still unclear—for example, we do not know whether the mechanisms that suppress Zn availability have a similar effect on Cd. Additionally, few studies have examined these relationships in natural soils that are not anthropogenically contaminated with Cd. Most studies use soils subject to industrial Cd contamination, or spike natural soils with Cd salts, which may behave differently from Cd naturally present as part of the mineral lattice or adsorbed onto organic matter in the soils. Many studies use hydroponic systems in growth trials, eliminating biological and adsorptive processes in the soil that complicate metal-plant interactions (e.g. Chan and Hale, 2004; Hart

et al., 2002). Soil Cd concentration can change uptake dynamics: for example, Xue and Harrison (1991) suggest that Cd and Zn are synergistic in high-Cd soils, but antagonistic in uncontaminated soils. Spiking soils with Cd for the purpose of the studying uptake may therefore introduce artifacts that do not represent normal growing conditions. Understanding the specific nature of Cd-Zn antagonism in natural crop-growing soils will be important to managing Cd uptake in wheat.

Since North Idaho is a productive wheat-growing region, it is important to determine this relationship in local soils. Phosphorus fertilization is a practice worth exploring for Cd management because it is universally used, easy to manipulate, and shows potential for indirectly affecting Cd uptake in wheat. Additionally, P guidelines for North Idaho were established over thirty years ago and may not account for more recent changes in soil pH and wheat varieties. Given the prospect of a premium for low-Cd wheat, P manipulation may be a financially viable strategy for farmers to reduce Cd uptake. With a better understanding of the relationship between P, Zn, and Cd in wheat, growers could use P management to control Cd accumulation.

Research Goals

This study aimed to address gaps in understanding of P, Zn, and Cd interactions in soil and wheat in order to inform nutrient management strategies that minimize Cd uptake in grain. We conducted a greenhouse experiment testing the effect of P rate on Cd and Zn uptake in wheat in an uncontaminated agricultural soil form Northern Idaho. We hypothesized that increasing P rate would increase Cd uptake and suppress Zn uptake; in other words, P rate would correlate positively with grain Cd:Zn ratio.

Methods

Experimental design and treatments

Soil selection: A Porrett ashy silt loam from Latah County, Idaho was used for this study because it had low P availability (Table 1.1), which allowed for testing various ranges of P fertilization. This soil was also selected for its low clay content, which is optimal for greenhouse potting experiments. Basic soil characteristics were measured by Norwest Agricultural Consultants (Kennewick, WA), a NAPT-PAP accredited laboratory, and are summarized in Table 1.1.

Table 1.1. Soil description and physicochemical characteristics of soil used in greenhouse study

Series	Porrett ashy silt loam
Family	Fine-silty, mixed, active Aquandic Epiaqualfs
Inorganic N (lbs/ac)	39
S (mg/kg)	2
pH	5.9
OM (%)	3.06
K (NaOAc) (mg/kg)	222
P (NaOAc) (mg/kg)	2.4
EDTA-Cd (mg/kg)	0.049
EDTA-Zn (mg/kg)	2.04
Total P (%)	0.063
Total Cd (mg/kg)	0.157
Total Zn (mg/kg)	68.1

Soil processing: Soil was collected from the top 6 inches of the profile. To preserve the structure and maintain drainage, large roots were removed and the soil was mixed gently to homogenize, but not sieved. The soil was air-dried and 485 g ($\pm$5 g) were weighed into 3 by 10-inch Deepots (D40, Stuewe and Sons, Corvallis, OR).

Fertilizer application: Norwest Agricultural Consultants analyzed the soil for inorganic N and acetate-extractable P. Based on soil test results, N fertilizer was applied to all pots in the form of urea at a recommended rate of 279 lbs N/acre to achieve 160 bushels per acre. Residual organic N was not included in this calculation. Including residual N, application rate would have been 219 lbs/acre. P was added as triple superphosphate (TSP, Bonide, Oriskany, NY). The TSP contained 51.4 mg/kg Cd (Washington State Department of Agriculture, 2017) (Table 1.2). Roberts (2014) reported that Cd concentrations in TSP range between less than 10 mg/kg and over 100 mg/kg. Dittrich and Klose (2008) reported a range of Cd concentrations between 37 and 73 mg/kg and a mean of 62 mg/kg from 11 TSP fertilizer samples. Based on these reported Cd concentrations, the fertilizer used in this experiment had an intermediate level of Cd contamination. Phosphorus was applied at four levels: none (P_0), the recommended rate based on soil testing of 40 lbs P_2O_5/acre (P_1), three times that rate (P_3), and five times that rate (P_5). Fertilizer rate calculations are shown in Appendix Table A.2. TSP contamination is summarized in Table 1.2. Fertilizer was ground,

weighed, and homogenized in each pot. Homogenized soils were lightly compacted into each pot.

Table 1.2. Cadmium and Zn content of TSP (Bonide, Inc.)

	Concentration (mg/kg)	Total added per pot (mg)		
		P_1	P_3	P_5
Cd	51.4	0.0016	0.0049	0.0081
Zn	631	0.0199	0.0597	0.0995

Plant culture: Each P treatment was replicated eight times for two sets of pots: one with plants and one without. Unplanted soils were included in the experiment to assess the effects of root exudates on soil metal availability. Pots were arranged randomly and re-randomized after every watering. The variety LCS Star was chosen because it is known for accumulating relatively high levels of Cd, which increased the likelihood that Cd concentrations were above the minimum detection level for the analytical instruments. Four seeds were planted in each pot 1 inch below the surface and thinned to 2 plants seven days after germination. Field capacity and permanent wilting point were assumed based on soil texture and the corresponding gravimetric water contents were calculated based on bulk density (Appendix Table A.1). The pots were watered every other day to maintain a weight between field capacity and halfway between field capacity and permanent wilting point. Four weeks after anthesis, watering was stopped and plants dried for two weeks.

Figure 1.1. Plants at seedling stage (left) and after drying (right)

Sample collection: Mature plants were separated into grain, shoots, and roots. The grain was ground in a mortar and pestle to coarse flour. Rhizosphere soil was collected by

shaking the roots to remove loosely adhering soil and gently beating them to remove clods greater than 2 cm in diameter. Each soil fraction was homogenized.
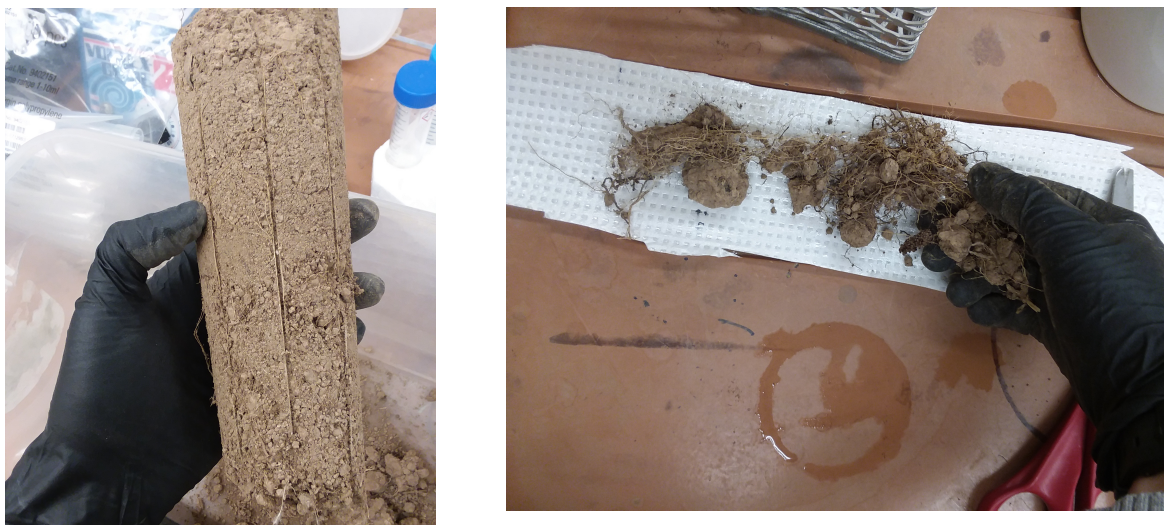
Figure 1.2. Root structure in core (left) and rhizosphere collection (right)

Analyses

Chemical analyses: Total metal concentrations from the grain samples were obtained using split digestion in nitric acid and aqua regia and ICP-MS analysis at Bureau Veritas (Vancouver, BC), an ISO/IEC 17025 accredited laboratory. Three replicate grain samples from the P_0 treatment were too small for that lab to analyze; those samples, along with known reference material ASTM SRM 1537 (tomato leaf), were digested in nitric acid and hydrogen peroxide according to the method described by Schulte (1985). Samples were pre-digested overnight in a 1:10 solid solution ratio in concentrated, trace metal grade HNO_3, heated to 60°C over one hour, oxidized with H_2O_2, and digested at 120 °C over 90 minutes. Digests were diluted, filtered through 0.45 µm polyether sulfone (PES) membrane filters, and analyzed for Cd, Cu, Zn, Mn, Fe, and P on an ICP-AES. Recovery rates are listed in Appendix Table A.3. Since recovery rates for Zn, Cd, Mn and P were 11-19% below the reported concentrations, we did not include these data in the statistical analyses. They are included in the complete results (Appendix Table A.4).

Soil samples from three replicates of the P_0 treatment of the unplanted soils were analyzed for total metals at Bureau Veritas using aqua regia digestion (1:1:1

$HNO_3:HCl:H_2O$) and ICP-MS analysis. Unplanted, bulk, and rhizosphere soils were analyzed for plant-available P, Cd, Zn, Fe, and Mn using the Mehlich 3 extraction, as described in Kovar and Pierzynski (2009). Fine-ground soil was prepared in a 1:10 solid solution ratio of Mehlich 3 extraction solution (0.2 M CH_3COOH, 0.25 M NH_4NO_3, 0.015 M NH_4F, 0.013 M HNO_3, 0.001 M EDTA), shaken for 5 minutes, filtered through 0.45 μm polyether sulfone (PES) membrane filter, and diluted to a 1:5 ratio. Extracts were analyzed on an ICP-AES for Cd, Zn, Mn, Fe, and P.

Statistical analyses: data was analyzed using one-way ANOVA and Tukey's HSD test to assess differences by P treatment in grain and soil metal concentrations.

Results

Grain yield

Grain yield increased significantly from P_0 and P_1 to P_3 and P_5 (Figure 1.3). Yield continued to rise, at a diminished rate, from P_3 to P_5. This indicates that P remained limiting despite the results of the initial nutrient availability test that suggested an application rate of 40 pounds per acre would meet crop requirements.
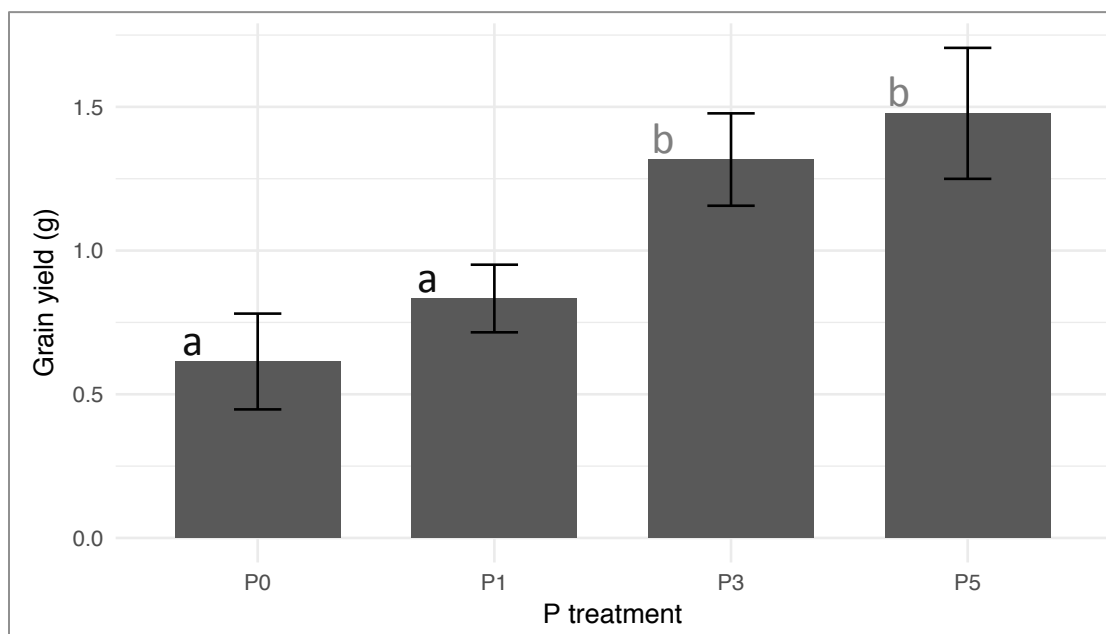
Figure 1.3. Mean grain yields from each pot for each P application rate (P_0 had no added P, P_1 was 1 times the P requirement, P_3 was 3 times the P requirement, and P_5 was 5 times the requirement). Error bars represent standard deviation and letters indicate significantly different groups ($p < 0.05$).

Grain metal uptake

Table 1.3 summarizes grain yield and grain Cd, Zn, Mn, Fe, and P concentrations. Table 1.4 shows total element uptake in grain. Table 1.5 shows the mean increase in total Cd and Zn absorbed by grain (compared to the P_0 treatment) as a percentage of total Cd and Zn added through fertilizer contamination. Increases in Cd and Zn uptake could be the result of TSP contamination, soil chemical, or biological factors; this percentage assumes the entire increase is due to contamination. Percentages are approximate because they rely on data from the Washington State Department of Agriculture, from which the particular batch of TSP used in this experiment could have deviated, and because of variations in how the fertilizer was distributed throughout the soil.

Table 1.3. Grain yield and element concentrations. Results for all samples and elements analyzed are listed in Appendix Table A.4.

	Yield (g)		Cd (mg/kg)		Zn (mg/kg)		Mn (mg/kg)		Fe (%)		P (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P-0	0.614	0.167	0.056	0.018	65.4	4.6	79.0	3.0	0.009	0.001	0.509	0.022
P-1	0.833	0.118	0.060	0.014	57.7	3.3	78.6	5.8	0.011	0.001	0.498	0.048
P-3	1.317	0.161	0.066	0.012	39.7	5.4	69.3	10.9	0.009	0.002	0.396	0.055
P-5	1.478	0.228	0.076	0.020	40.7	6.0	72.1	5.5	0.007	0.000	0.418	0.071

Table 1.4. Total element uptake in wheat grain.

	Cd (µg)		Zn (mg)		Mn (mg)		Fe (mg)		P (mg)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
P0	0.034	0.019	0.045	0.011	0.055	0.012	0.059	0.009	3.515	0.653
P1	0.052	0.013	0.050	0.004	0.068	0.008	0.093	0.009	4.317	0.494
P3	0.087	0.019	0.052	0.004	0.091	0.015	0.113	0.024	5.137	0.165
P5	0.112	0.033	0.060	0.010	0.106	0.016	0.107	0.017	6.091	0.950

Table 1.5 shows the proportion of Cd added through contamination accumulated by the grain (assuming all the additional Cd absorbed at each P application rate was due to contamination). This proportion was calculated using $\frac{Grain\ Cd_i - Grain\ Cd_0}{Soil\ Cd_{added}}$, where Grain Cd_i is average total Cd content in grain at a given P level, Grain Cd_0 is baseline grain Cd content at treatment P_0, and Soil Cd_{added} is the mass of Cd added through TSP. The proportion remained at 1 percent for all P levels.

Table 1.5. Percentages of Cd added through TSP absorbed by grain

	P_1	P_3	P_5
Grain Cd_i – Grain Cd_0 (µg)	0.0181	0.0529	0.0783
Cd in TSP added to each pot (µg)	1.62	4.86	8.10
Percent of added Cd absorbed by grain	1.11%	1.09%	0.966%
Grain Zn_i – Grain Zn_0 (µg)	10.3	12.5	20.4
Zn in TSP added to each pot (µg)	19.9	59.7	99.5
Percent of added Zn absorbed by grain	51.8%	20.9%	20.5%

Grain P concentration decreased as P application rate increased (Table 1.3, Figure 1.4), decreasing significantly between treatments P_1 and P_3 ($p < 0.05$). Grain Zn exhibited a similar pattern, with a significant decrease between P_1 and P_3. Grain Cd rose with P treatment, but not significantly ($p < 0.195$). Cd/Zn ratio rose with P application, increasing significantly between P_1 and P_3.
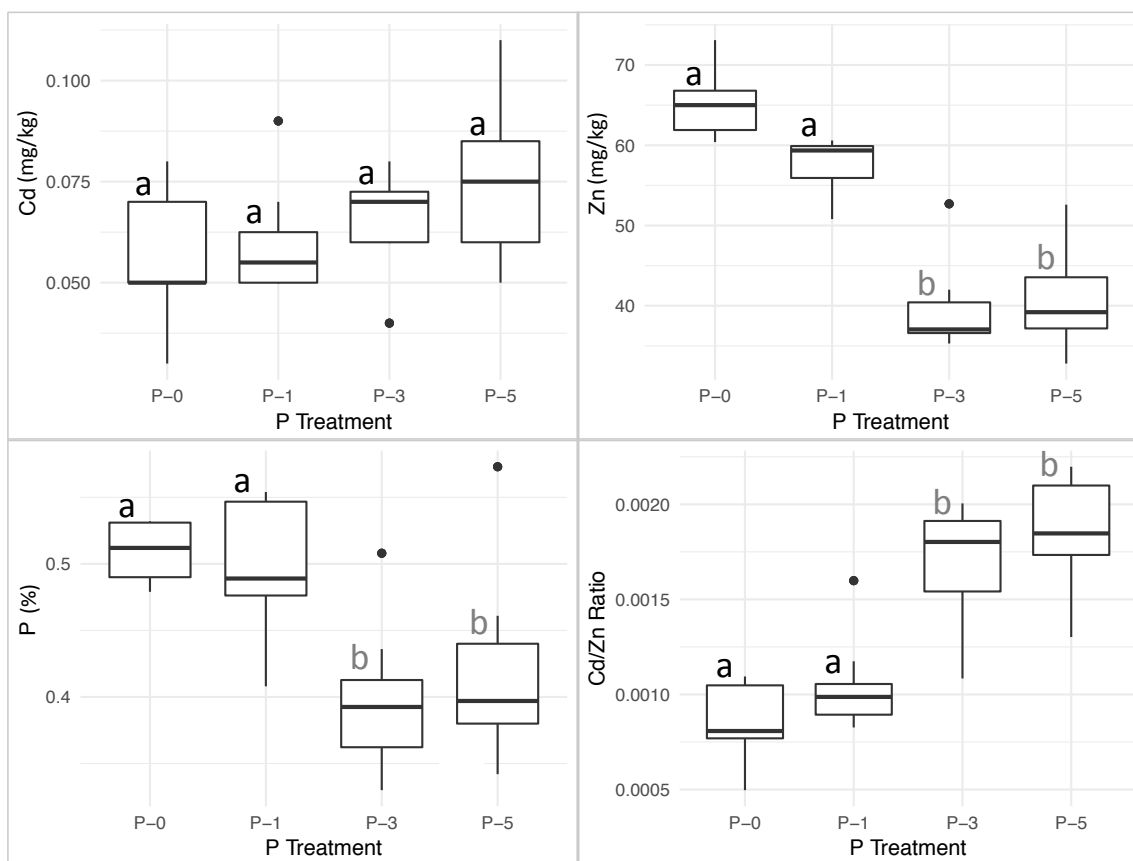
Figure 1.4. Grain Cd, Zn, and P concentrations. Horizontal lines represent quartiles, vertical lines represent 1.58 times the interquartile range (which represents approximately a 95% confidence interval about the median. See McGill et al., 1978), dots represent outliers, and different letters show significantly different groups ($p < 0.05$).

Figure 1.5 shows total grain uptake of Cd and Zn. Total Cd accumulation increased significantly between treatments P_1 and P_3 and Zn increased significantly between P_0 and P_5 ($p < 0.05$).
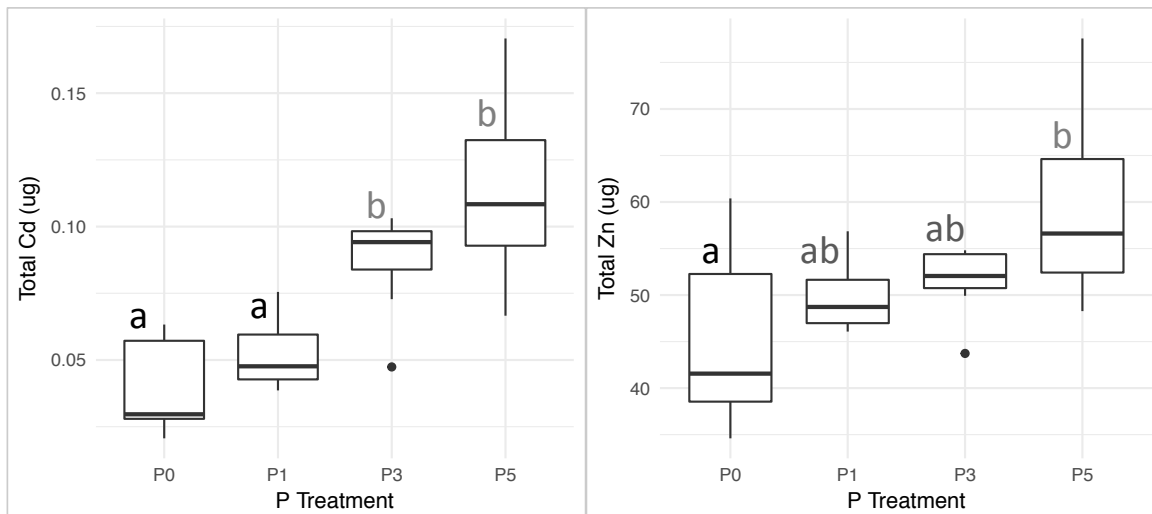
Figure 1.5. Total Cd and Zn accumulation in grain. Treatments that do not share a letter are significantly different ($p < 0.05$).

Grain Cd concentrations ranged between 0.03 and 0.11 mg/kg and treatment means ranged between 0.056 and 0.076 mg/kg, all below the FAO maximum of 0.2 mg/kg (FAO, 2015). Treatment means ranged between 64% and 78% of the distribution of Cd concentrations measured in 969 samples across Idaho (Figure 1.6).
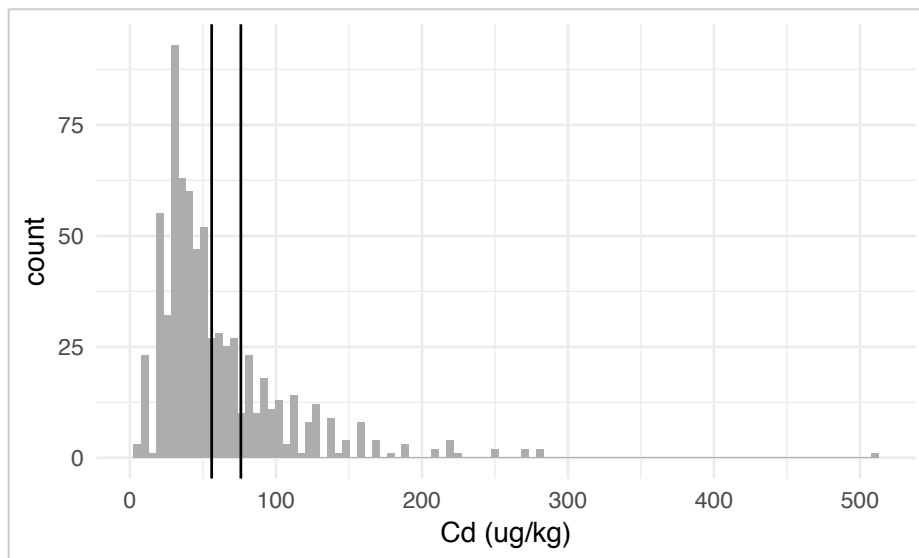
Figure 1.6. Grain Cd concentrations in Idaho (see Chapter 2). Vertical lines represent the range of the mean Cd grain concentrations from each treatment in this experiment.

Despite the rise in Cd and fall in Zn grain concentrations as P application increased, grain Cd and Zn did not show a strong negative correlation (p > 0.05) (Figure 1.7). However, within each treatment grain Zn and Cd show positive correlation. Although statistical inference should not be drawn from replicates within treatments, this pattern in Cd and Zn accumulation suggests that, holding P rate constant, Zn and Cd were synergistic rather than competitive.
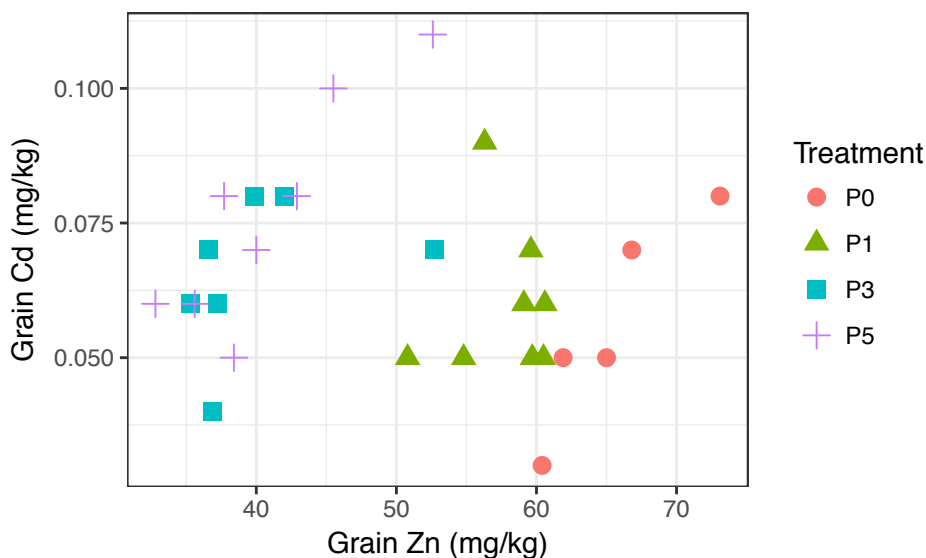
Figure 1.7. Grain Cd concentration vs. grain Zn concentration.

Soil metal concentrations

Table 1.6 shows total soil metal concentrations in unplanted soils from the P_0 treatment, and Table 1.7 shows soil pH and Mehlich-extractable P and metals. Soil pH was lowest in the unplanted soil and did not change with P treatment. pH was slightly higher in the planted soil and rose with P application in both fractions, though not significantly. Available Mn concentration significantly decreased (p < 0.05) in the rhizosphere, indicating that soil Mn was either fixed or depleted. Mehlich-extractable Cd rose slightly with P application in the soil fractions not in close contact with the roots, i.e. the unplanted and bulk soils. In the rhizosphere, it was highest at P_0 but did not increase or decrease significantly with P. As with Cd, Mehlich-extractable Zn rose slightly with P application in the unplanted and bulk soils, but was lower in the rhizosphere and showed no clear trend with P rate. Mehlich P increased with P application in all soil fractions and was lower in the rhizosphere than the unplanted and bulk fractions.

Table 1.6. Total soil metal concentrations in unplanted soils from the P_0 treatment (n = 3).

Total soil metals	Mean concentration	SD
Cd (mg/kg)	0.157	0.0174
Zn (mg/kg)	68.1	0.0132
Mn (mg/kg)	1500	0.0122
P (%)	0.0630	0.0030
Fe (%)	2.43	0.0193

Table 1.7. Soil pH and Mehlich-extractable metal concentrations. Results for all elements and samples analyzed are listed in Appendix Table A.5.

	P level	pH		Cd (mg/kg)		Fe (mg/kg)		Mn (mg/kg)		P (%)		Zn (%)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Unplanted	P_0	5.41	0.18	0.049	0.004	240.3	16.00	90.73	14.19	28.93	2.37	2.04	0.21
	P_1	5.35	0.10	0.051	0.002	244.4	8.43	97.28	6.56	33.01	2.00	2.12	0.11
	P_3	5.34	0.15	0.057	0.003	251.1	9.65	93.74	2.72	51.88	6.11	2.39	0.09
	P_5	5.42	0.13	0.057	0.005	252.5	11.71	95.52	8.99	52.83	8.56	2.37	0.25
Bulk	P_0	5.65	0.35	0.062	0.007	256.3	14.68	139.51	14.52	40.40	8.12	2.25	0.27
	P_1	5.73	0.23	0.065	0.005	260.0	8.94	143.33	16.20	43.71	9.80	2.33	0.15
	P_3	6.06	0.22	0.062	0.006	244.5	22.46	120.09	27.44	46.19	7.34	2.36	0.18
	P_5	6.00	0.21	0.070	0.008	269.7	19.14	137.31	13.61	52.27	12.08	2.50	0.20
Rhizosphere	P_0	5.40	0.29	0.056	0.006	259.3	31.21	113.36	15.50	26.06	1.58	2.04	0.23
	P_1	5.69	0.18	0.049	0.005	247.5	24.46	100.73	14.58	26.37	1.68	1.82	0.28
	P_3	5.67	0.10	0.054	0.005	262.3	13.38	93.52	13.15	37.71	2.69	2.07	0.26
	P_5	5.89	0.18	0.053	0.005	256.5	23.48	85.99	13.72	39.29	3.45	1.92	0.23

Figure 1.8 shows Mehlich-extractable soil metals and Figure 1.9 shows the relationship between Mehlich P and grain Cd concentration.
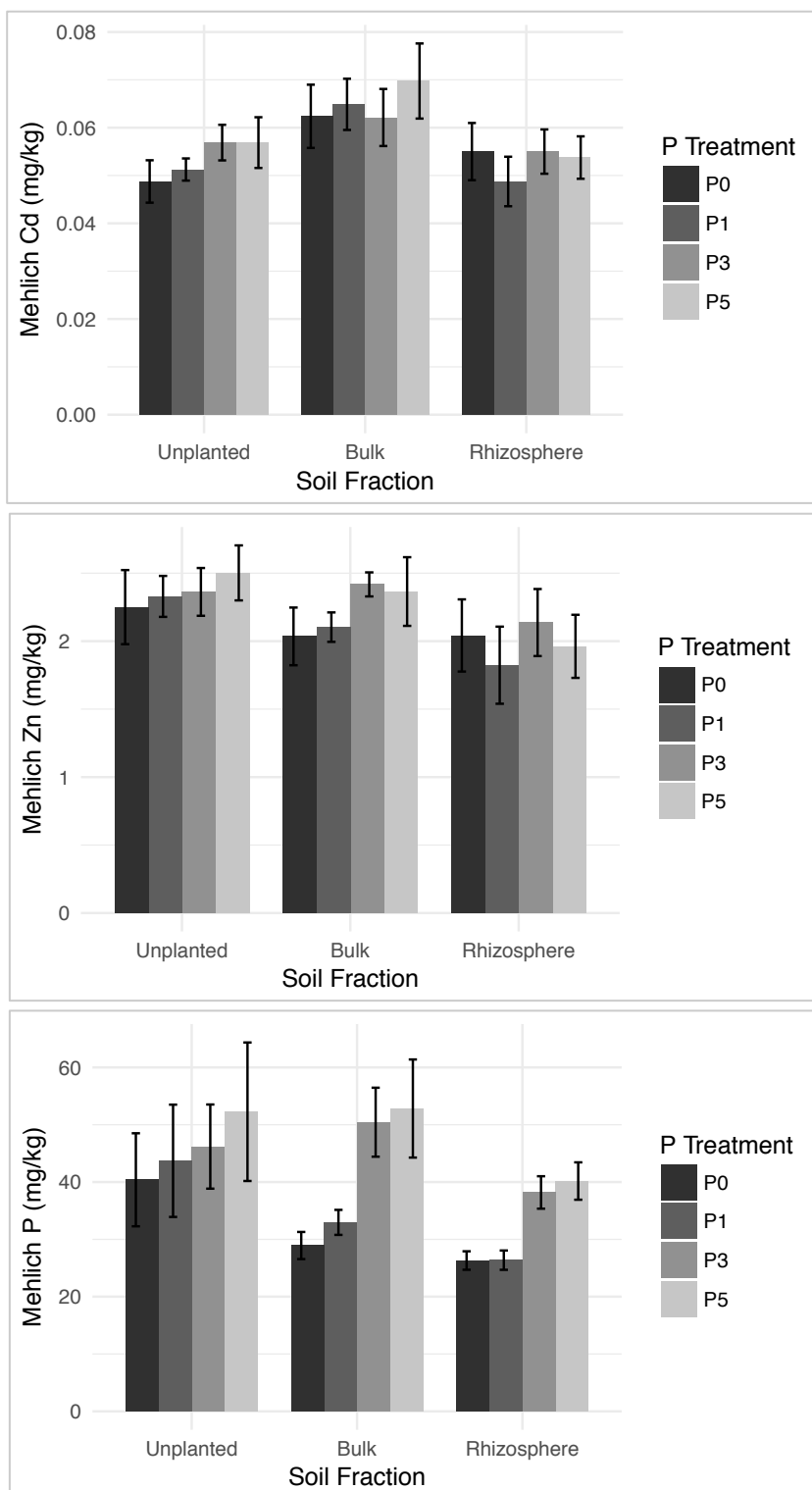
Figure 1.8. Mehlich-extractable Cd, Zn, and P in rhizosphere, bulk, and unplanted soils in different P treatments. Error bars show standard deviations.
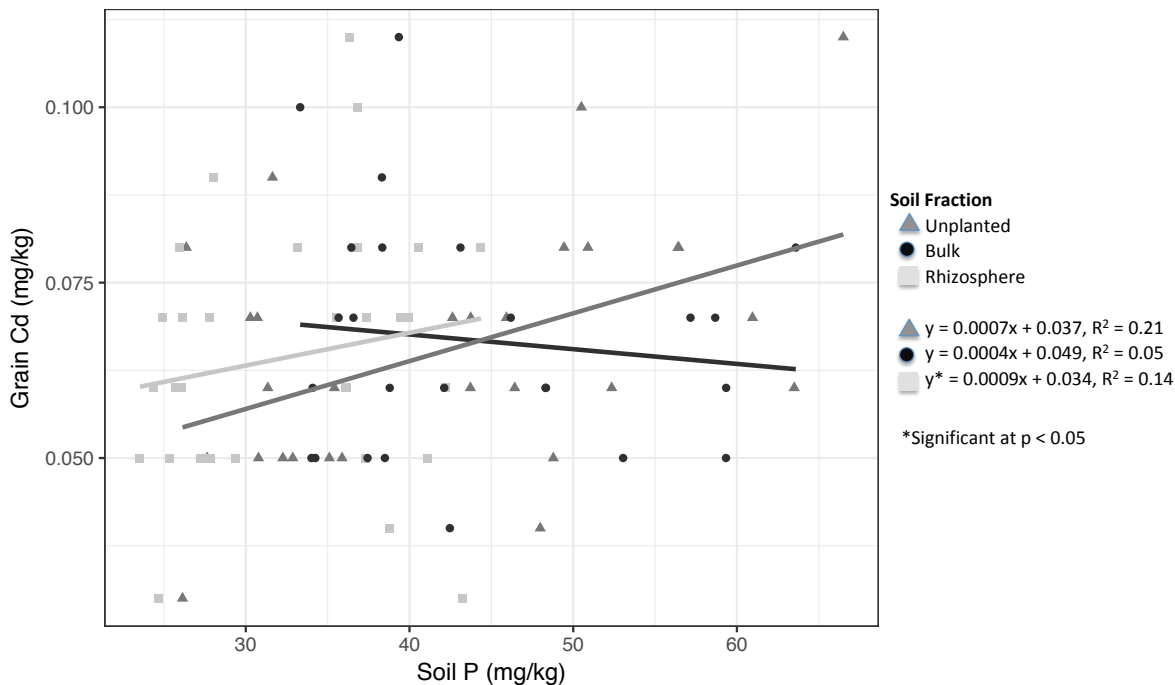
Figure 1.9. Grain Cd vs. Mehlich-extractable P

While grain Cd concentration did not increase significantly with P treatment, there was a weak but significant ($p < 0.05$) positive correlation between available P in the rhizosphere and grain Cd.

Bioaccumulation factor (BAF) is typically calculated as the ratio of contaminant concentration in the plant to its total soil concentration (Strawn et al., 2015).

$$BAF = \frac{[Metal\ in\ plant]}{[Total\ metal\ in\ soil]}$$

Here, however, the plant-available fraction of soil Cd (Mehlich extractable) was used instead of total soil Cd to assess how plants' capacity to absorb *available* Cd changed with P treatment. Cd BAF increased with P treatment, but not significantly ($p > 0.05$ for all soil fractions), but Zn BAF decreased significantly (Figure 1.10).
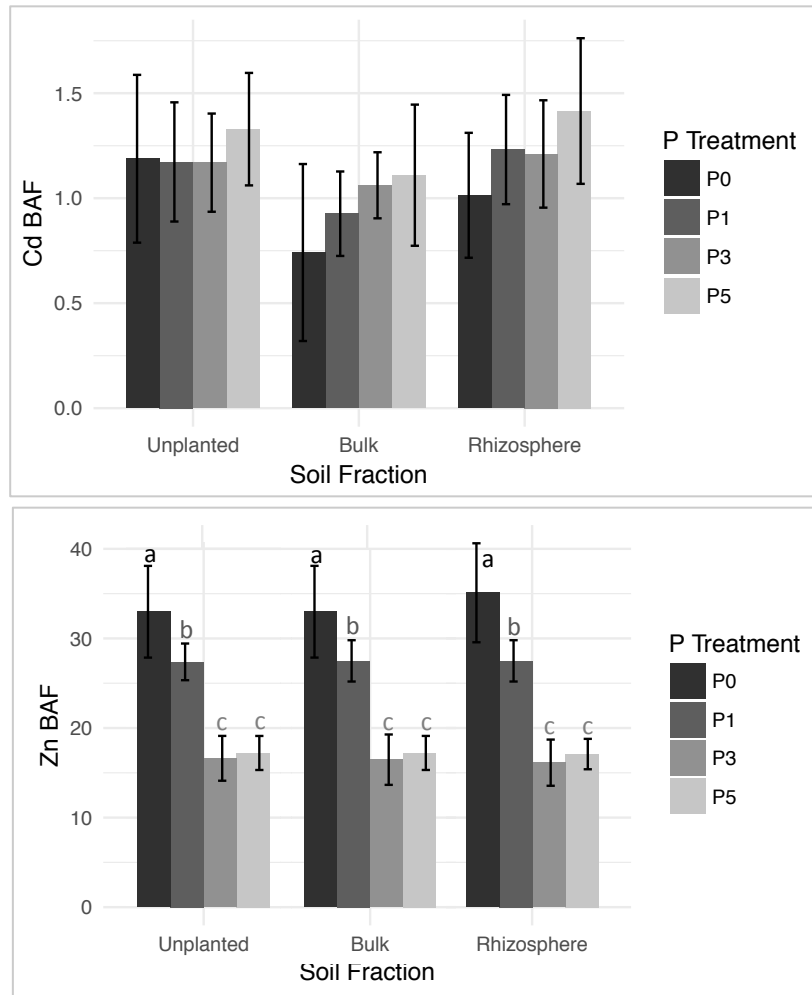
Figure 1.10. Cd and Zn bioaccumulation factor. Differences in Cd BAF between P treatments were insignificant ($p > 0.05$) for each soil fraction, but significant for Zn BAF.

Discussion

Grain Cd concentrations ranged between 0.03 and 0.11 mg/kg and treatment mean Cd concentrations ranged between 0.056 and 0.076 mg/kg, all below the FAO maximum allowable Cd in wheat grain of 0.2 mg/kg (FAO, 2015). The grain Cd concentration is relatively high for Idaho wheat, especially considering the low soil Cd and abundant Zn. Treatment means ranged between 64% and 78% of the distribution of Cd concentrations described in Chapter 2 of this thesis. However, since LCS Star is thought to be a high-uptake variety (personal communication, Chen, 2017), the Cd concentrations from the greenhouse experiment are not out of the expected range. There were some signs of water stress in the plants over three days during the jointing phase of the greenhouse trial, which may have limited biomass growth and concentrated Cd in the plant tissues.

Grain Cd concentration increased with P application rate, but not significantly ($p > 0.05$). Mean grain Cd concentration increased by 4, 10, and 20 mg/kg (7, 18, and 36 percent) from P_0 to P_1, P_3, and P_5, respectively. This shows that, despite the significant increase in total Cd accumulation, P application did not significantly change the plants' capacity to mine soil Cd on a per-mass basis. Grain Zn decreased significantly from P_0 and P_1 to P_3 and P_5. Despite these trends, grain Zn concentration did not correlate with grain Cd, suggesting Cd-Zn antagonism was not a major mechanism. While Cd/Zn ratio did increase significantly with P rate, as predicted, this was apparently due to Zn suppression from P rather than increased Cd uptake. Grain Zn content had no clear relationship with grain Cd, providing little evidence for antagonism. Studies have observed Zn-Cd competition primarily when Zn is deficient (e.g. Adiloglu, 2002; Oliver et al., 1994, Grant and Bailey 1998, Zhu et al., 2003). EDTA-extractable (Mehlich) Zn in the unplanted P_0 soil was an ample 2 mg/kg. Zinc deficiency in wheat typically occurs at soil concentrations between 0.4 and 0.6 mg/kg by DTPA extraction, which typically yields slightly lower values than EDTA (Mahler et al., 1981, Bakircioglu et al., 2011). The Mehlich 3 extraction uses EDTA, which is why this measure of plant availability was used instead of DTPA. Sufficient Zn in the soil therefore may have prevented Cd-Zn competition for uptake.

Mehlich-extractable Cd rose slightly with P application in the soil fractions not in close contact with the roots, probably due to contamination. In the rhizosphere, however, it was highest at P_0 and decreased slightly or remained the same across P treatments. Similarly, Mehlich-extractable Zn rose with P rate in the bulk and unplanted soils but was lower in the rhizosphere and did not rise (Figure 1.8). The lower Cd levels in the rhizosphere probably resulted from a combination of fixation and depletion, although the extent to which each took place is difficult to determine without a complete accounting of Cd in roots and shoots, which were not analyzed. Wheat has been observed to store a relatively large portion of the toxic metals it absorbs in its roots, and, according to Liu et al. (2009), less than 1 percent in the seed. Total Cd uptake therefore was probably substantially more than what is shown here, meaning rhizosphere depletion could have been significant.

Rhizosphere available Zn levels, on the other hand, appear to have decreased primarily due to P-induced fixation either in the soil, or through sequestration in the plant. Depletion is unlikely, since grain Zn declined with increasing P. These results are similar to those found by

Austruy et al. (2014), who observed P application induced Zn fixation in rhizosphere, but not bulk, soils. They suggested P-metal fixation is enhanced when root exudates solubilize rhizosphere P, making it available to complex with metals. Previous research supports our observation that P addition resulted in greater fixation of Zn than Cd. Kandah (2004) found low-grade phosphate adsorbed greater quantities of Zn than Cd, which the author attributes to Zn's smaller ionic radius. In solution together, Zn and Cd competed for adsorption, but the presence of Zn decreased Cd adsorption more than Cd decreased Zn adsorption. Lambert et al. (2007) observed that water-extractable soil Cd increased with P application rate while Zn concentration decreased, indicating that Zn was being adsorbed or precipitated by P to a greater degree than Cd was. Bolan et al. (2014) report that phosphate addition can cause soluble Cd-P complexes to form, decreasing Cd adsorption and potentially making Zn more competitive for adsorption. The possibility that P preferentially immobilizes Zn could mean that in Zn-deficient soils, P amendment could increase Cd's competitiveness for uptake. While P fertilization did not substantially increase Cd accumulation in this experiment, P-induced Cd uptake could pose a greater risk in low-Zn soils.

Cadmium addition through P fertilizer contamination may have been an important driver in increasing Cd uptake. The increase in Cd uptake with P treatment was about 1 percent of the Cd added through TSP fertilization at every P level (Table 1.5). The fact that this proportion was constant across P treatments suggests that other proposed mechanisms for P-induced Cd uptake, such as P-induced changes in soil chemistry or microbial community, did not substantially change the plants' capacity to absorb added Cd. If, for example, added P inhibited mycorrhizal sequestration of Cd, the proportion of added Cd absorbed would be expected to increase with P amendment rate. The fact that the increase in grain Cd increased directly in proportion to amount of fertilizer Cd added to the soil suggests that a secondary P mobilization or immobilization process did not occur.

On the other hand, the bioaccumulation factor for Cd increased with P addition, which could imply that some mechanism did cause the grain to increase the proportion of available Cd it absorbed as P application rate increased. An increase in BAF implies that grain Cd concentration increased faster with P rate than did the amount of available Cd added to the soil, as shown in Figure 1.11. The "rate" of grain concentration increase was greater than that of the soil, suggesting a change in soil Cd availability exogenous to Cd addition through

fertilizer. The fact that grain concentration increased relative to soil could indicate secondary Cd mobilization processes increased the uptake of added Cd. However, it is difficult to assess whether plant accumulation increased disproportionately to soil availability without accounting for tissue concentrations in the rest of the plant. A more thorough assessment of the role of Cd contamination on grain concentration response would require a complete analysis of the plant tissue to gain a better understanding of the mass balance of Cd.
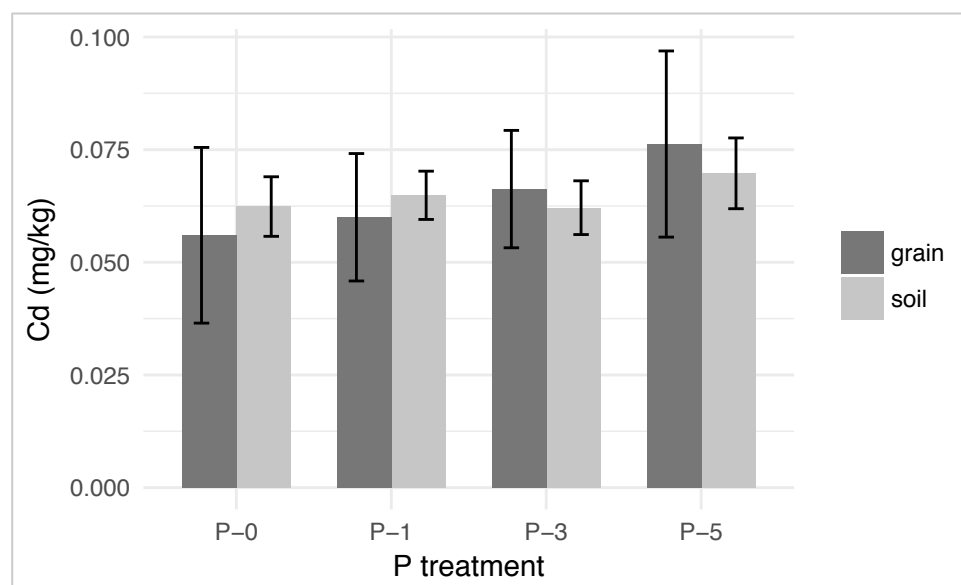
Figure 1.11. Cd concentrations in grain and soil (bulk fraction, Mehlich-extractable). Error bars show standard deviations.

Conclusion

Increasing P application rate was associated with a statistically insignificant rise in grain Cd concentration. It was related to a significant increase in grain Cd:Zn ratio, but this was due more to Zn suppression than increased Cd uptake. Quintupling the P rate resulted in a small difference in mean grain Cd concentration: mean grain Cd increased 4, 10, and 20 μg/kg (7, 18, and 36 percent) from P_0 to P_1, P_3, and P_5, respectively. This experiment did not provide statistically significant evidence that P application increases Cd uptake. Small changes in Cd uptake could be valuable to growers aiming for an ultra-low Cd standard, although these results indicate that this may require a large reduction in P fertilizer that could lower yields. However, the effects of P application rate on Cd uptake through P-induced Zn suppression may have been more pronounced in a Zn-deficient soil, such as those that are common on Idaho's Snake River Plain, another important wheat growing region.

Cadmium contamination appeared to be an important mechanism for uptake, but perhaps not the only one, considering the rising Cd bioaccumulation factor and fluctuations in rhizosphere available Cd with P treatment. P-induced Zn fixation, mycorrhizal suppression, and increased plant growth may have played supplementary roles in determining Cd uptake. While P-induced fixation of Cd was ambiguous, P application clearly fixed soil Zn in the rhizosphere or roots. Phosphorus complexes formed in the soil or roots after fertilization likely had a greater affinity for Zn than Cd.

The results of this experiment suggest that altering P application is not a cost-effective strategy to lower Cd uptake in soils with high Zn availability. Manipulating P application rate may have more significant effects on Cd uptake in Zn-deficient soils. Future studies should address interactions between P, Zn, and Cd in low-Zn soils to investigate the possibility of Cd-Zn competition under P-induced Zn deficiency. Research should also account more thoroughly for mechanisms of P-induced Cd uptake in order to parse the role of soil fixation, microbial activity, and plant physiology on Cd and Zn availability.

Chapter Two: An Exploratory Analysis of How Variety and Element Relationships Affect Cadmium Uptake in Wheat

Introduction

Overview

Controlling cadmium (Cd) uptake in wheat is a public health priority. Cd is a toxic contaminant: it serves no biological purpose in any organism, and in relatively small quantities causes health problems in humans such as kidney failure and bone demineralization. Wheat, which accumulates relatively high levels of Cd among cereal plants, is a potential source of Cd in the human diet (Grant et al., 2013). It is important to understand how environment and management impact Cd uptake in wheat in order to control its incidence in the food supply.

Two types of factors control Cd uptake in wheat: 1) soil's ability to retain Cd, and 2) plants' ability to absorb and internally translocate Cd. The total soil concentration of Cd in soil may be a less important determinant of plant uptake than soil properties that influence its availability to plants. Metal bioavailability is often represented by the fraction extractable by DTPA, a chemical extractant that mimics root exudates (Bakircioglu et al., 2011). Mineral sorbents like manganese oxides may help soils retain Cd, while organic matter can be up to thirty times as effective as mineral soil in adsorbing trace metals (Baize et al., 2009). Soil pH, which affects mineral solubility and soil's capacity to adsorb Cd on exchange sites, may be the most important determinant of soil Cd availability (Lambert et al., 2007). The second category of factors that affect Cd uptake in plants includes biological characteristics like plant physiology, microbial community, and levels of other trace metals that compete or are synergistic with mediated or active uptake of Cd. Zinc (Zn), which is similar in size and charge to Cd, is widely observed to compete with Cd for transport proteins in the plant (Hart et al., 2002).

In this chapter, relationships between soil properties, metal concentrations in soil and wheat, and Cd uptake are investigated. Understanding inter-elemental relationships can guide management strategies for reducing Cd uptake. Management techniques, particularly

fertilizer application, influence the availability of a variety of nutrients and trace metals, which in turn affect Cd availability. Cultivar selection is another aspect of management with the potential to influence Cd uptake. Physiological differences between cultivars may include differences in transport proteins, which exhibit a variety of competitive and synergistic relationships between trace metals. For example, Cd and Mn are thought to compete for plant absorption via the NRAMP family of transporters, while the ZIP family exhibits competitive uptake between Cd and Zn (Fontanili et al., 2016). Such observations are useful in fields such as ionomics, which investigates biological processes through total elemental concentrations. Understanding these competitive dynamics could aid in variety selection based on available soil nutrients. With a better understanding of inter-elemental relationships in soil and wheat, researchers can identify management techniques that minimize the occurrence of Cd in the food supply.

Research Goal

In an effort to clarify the relationship between grain Cd accumulation and the availability of other trace metals and nutrients, metal concentrations in soil and wheat over four seasons from growers across Idaho were measured. Soil and grain metal concentrations are summarized, and grain Cd concentration is modeled as a function of soil metals, grain metals, and variety. Because the project objectives varied each year, sampling sites and strategies varied as well. While this sampling makes data interpretation difficult, characterizing the overall properties provides some insight into trends in soil and grain Cd accumulation. This information can be used for screening and developing future research projects to better understand how soil and wheat variety factors affect Cd uptake.

Methods

Sampling

Location: Sampling locations varied from year to year (Table 2.1). In 2013, the first year of the study, composite grain samples were obtained from a grain buyer that collected them from Southern Idaho growers as a part of their Cd screening program. No soil samples were taken in 2013. In 2014, soil and grain were sampled from University of Idaho small grain variety test plots at several Southern Idaho locations. In 2015, the study expanded to several Northern Idaho research plots. In 2016, samples were taken as part of an effort to find

low Cd uptake varieties for piloting field to mill delivery of low Cd grain. Since sampling locations were often chosen based on location of university test plots and association with growers, they do not control for or represent environmental variation such as climate and soil type in a systematic way.

Table 2.1. Sampling years and locations

Year	Sampled	Location
2013	Soil	
	Grain	Ashton, Idaho Falls, Kimberly, Parma, Ririe, Rockland, Rupert, Soda Springs
2014	Soil	Aberdeen, Kimberly, Tetonia
	Grain	Aberdeen, Kimberly, Tetonia
2015	Soil	Ashton, Bonners Ferry, Genesee, Moscow, Nezperce, Moscow, Soda Springs, Tammany, Tensed
	Grain	Aberdeen, Ashton, Bonners Ferry, Genesee, Idaho Falls, Kimberly, Moscow, Nezperce, Moscow, Ririe, Rupert, Soda Springs, Tammany, Tensed
2016	Soil	Parma, Nezperce, Rexburg, Southwick, Tensed
	Grain	Parma, Nezperce, Rexburg, Southwick, Tensed

Soil: 4 to 7 soil samples were taken from each location at depths of 0-6, 6-12, and 12-24 inches (or, in a few cases, 12-18 and 18-24 inches). Samples were taken at different landscape positions across each wheat field to account for variability in soil properties.

Grain: grain from 205 wheat varieties was sampled from 34 locations over 4 years. The number of years and locations where grain cultivars were sampled varied. Some locations had many replicates of one variety, some had several replicates of several varieties, and some had one replicate each of many varieties. Some samples were taken from individual groups of plants, while some were composited from grain bins. In 2016, varieties were selected for testing that were known to have low Cd accumulation, which may bias results for soils in which those samples were grown. Because of the sampling strategy's inconsistencies, the analyses described below are exploratory, and should be used to guide further research.

Chemical Analyses

Soil: Soils from three growing seasons were measured for pH, electrical conductivity, total elemental concentrations, and DTPA-extractable cadmium, zinc, manganese, copper, and iron. DTPA extraction is a common measure of plant-available trace metals (Haynes and Swift, 1983). Soils were prepared according to the method described by Reed and Martens (1996) in a 1:2 solid-solution ratio of DTPA extraction solution (0.005 M DTPA, 0.01 M $CaCl_2$, 0.1 M TEA), shaken for 2 hours, centrifuged at 2,000 rpm for 10 minutes, filtered through 0.22 μm polyether sulfone (PES) membrane filters, and diluted to a 1:5 ratio. Extracts were analyzed on an ICP-AES for Cd, Cu, Fe, Mn, and Zn. Total metal concentrations of soils were measured by Bureau Veritas (Vancouver, BC), an ISO/IEC 17025 accredited laboratory. The soil samples were digested in aqua regia, followed by analysis on an ICP-MS. For most soils, DTPA extractions were only performed on the top six inches of soil, but in a few, analyses were performed on subhorizons.

Grain: Cd concentrations in grain samples from 2014-2016 were determined by Bureau Veritas. Grain samples were digested in aqua regia and underwent total elemental analysis on an ICP-MS. Samples from the 2013 growing season were analyzed for Cd content by the grain milling company in their food quality laboratory.

Statistical Analyses

Principal components analysis (PCA) was performed on the soil and grain datasets to investigate their structure. PCA groups variables into linear combinations, or components. The first principal component is the combination associated with the greatest variance in the dataset, and each subsequent PC is associated with progressively less variance. Subsequent PC's must be orthogonal to the preceding PC's. Grouping the variables this way reduces the number of dimensions and guarantees they will not be collinear (Powell and Lehe, 2015). While PC's can themselves be used as regression terms, they can be difficult to interpret as real-world variables. In this analysis PCA was used to illustrate important ways in which soil and grain varied, and how those variations were distributed regionally. Samples without measurements for potentially important variables were excluded from this analysis, as were variables measured in less than half the samples. Soil measurements were averaged for each site by depth. Mean annual precipitation and air temperature for each location were included

in the analysis. These data were taken from the NOAA weather station nearest each sampling location (NOAA, 2010).

Wheat physiological attributes such as root exudate composition and expression of transporters can impact cadmium accumulation. (Chan and Hale, 2004). These attributes may affect antagonisms and synergisms in uptake between elements, as in the case of transporter proteins with high affinities for certain metals. Since these properties vary by variety, we selected the ten cultivars with the greatest sample sizes (Brundage 96, LCS Artdeco, Norwest 553, UI Pettit, UI Platinum, UI Silver, UI Stone, UI-WSU Huffman, and WB Junction) and assessed correlations between metals in each. Not all varieties were grown in all soils, and not all soils were represented in every variety. Comparisons between varieties may be confounded by environmental factors—differences in metal concentrations may simply reflect differences such as climate or soil type. These comparisons are therefore exploratory and intended to guide future research.

Results and Discussion

Summary of Metal Concentrations in Soil and Grain

95% of sites had total soil Cd concentrations below 1 mg/kg. The highest total Cd concentration sampled was 1.76 mg/kg, which is much lower than typical concentrations in contaminated sites used for agriculture (e.g., 3.15 mg/kg in Rehman et al. (2015), 10.36 mg/kg in Nan et al. (2002), 6.84 mg/kg in Chaney et al. (2006)). Table 2.2 summarizes soil pH, electrical conductivity, DTPA-extractable and total Cd, Cu, Fe, Mn, and Zn, and total soil P.

Table 2.2. Soil pH, EC, and metal concentration

	pH	EC	DTPA-Cd (mg/kg)	DTPA-Cu (mg/kg)	DTPA-Fe (mg/kg)	DTPA-Mn (mg/kg)	DTPA-Zn (mg/kg)
Mean	6.47	241.79	0.20	4.72	74.05	30.92	2.70
Min.	4.12	24.60	0.01	0.24	0.74	0.66	0.04
Max.	9.09	1897.00	1.76	68.95	518.70	231.15	22.47
St. dev.	1.30	206.88	0.28	7.77	105.39	45.11	3.24
		Total P (%)	Total Cd (mg/kg)	Total Cu (mg/kg)	Total Fe (%)	Total Mn (mg/kg)	Total Zn (mg/kg)
Mean		0.08	0.46	20.09	2.78	570.33	84.27
Min.		0.04	0.09	6.50	1.08	96.00	37.50
Max.		0.81	1.85	65.82	4.94	2539.00	128.30
St. dev.		0.05	0.24	8.38	0.89	245.03	17.41

Figures 2.1 and 2.2 show the distribution of total and DTPA-extractable Cd concentrations in soil.
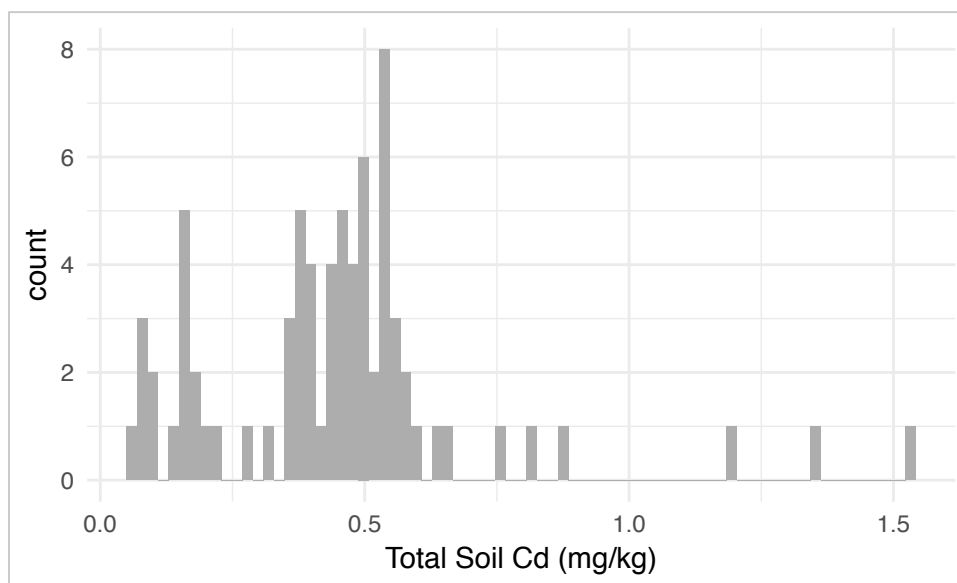
Figure 2.1. Total soil cadmium concentrations (n = 74)
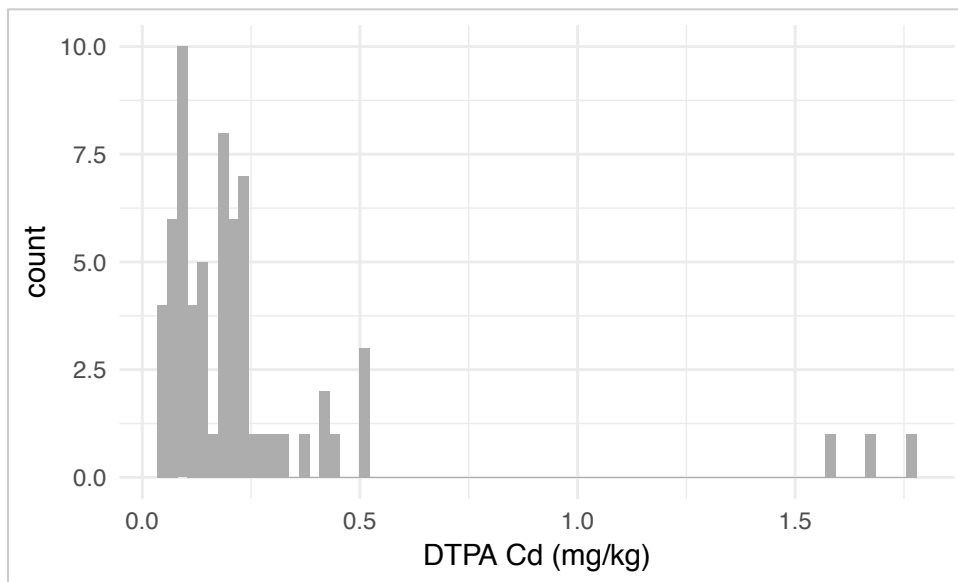
Figure 2.2 DTPA-extractable and total soil Cd concentrations (n = 74)

Figure 2.3 shows the geographic distribution of total and DTPA-extractable Cd concentrations in soil. The total soil Cd concentration of sites associated with grain concentrations over the United Nations Food and Agricultural Organization's limit of 0.2 mg/kg (FAO, 2015) was 0.5 mg/kg; DTPA-extractable Cd concentrations were more varied at those sites. These sites were located in Ashton, Bonners Ferry, Rexburg, and Soda Springs.
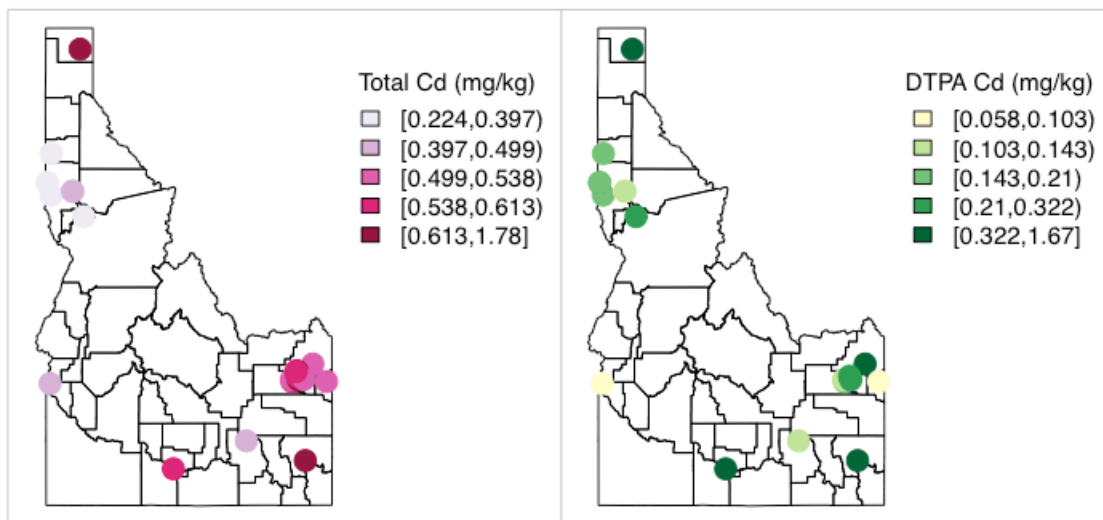
Figure 2.3. Total (left) and DTPA-extractable (right) Cd concentrations in soil

Climatic differences in Northern and Southern Idaho produce distinct soil characteristics: in North Idaho higher precipitation tends to result in more acidic, leached

soils with higher organic matter. These properties can lead to differences in Cd availability. On average, samples from North Idaho had significantly lower pH ($p < 0.05$, Figure 2.4), slightly higher DTPA-extractable Cd ($p > 0.05$, Figure 2.5), and significantly lower total Cd ($p < 0.05$, Figure 2.5). Soil pH and DTPA-Cd concentration had a weak negative relationship (Figure 2.6).
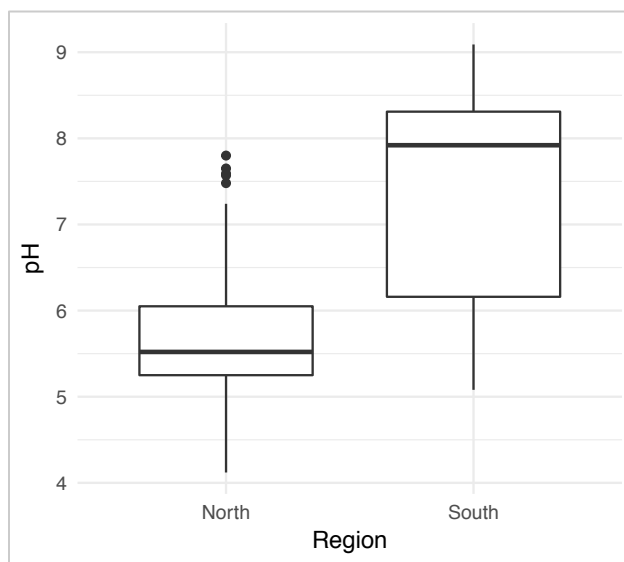
Figure 2.4. pH in Northern and Southern Idaho. Horizontal lines represent quartiles, vertical lines represent 1.58 times the interquartile range (which represents approximately a 95% confidence interval about the median. See McGill et al., 1978), and dots represent outliers.
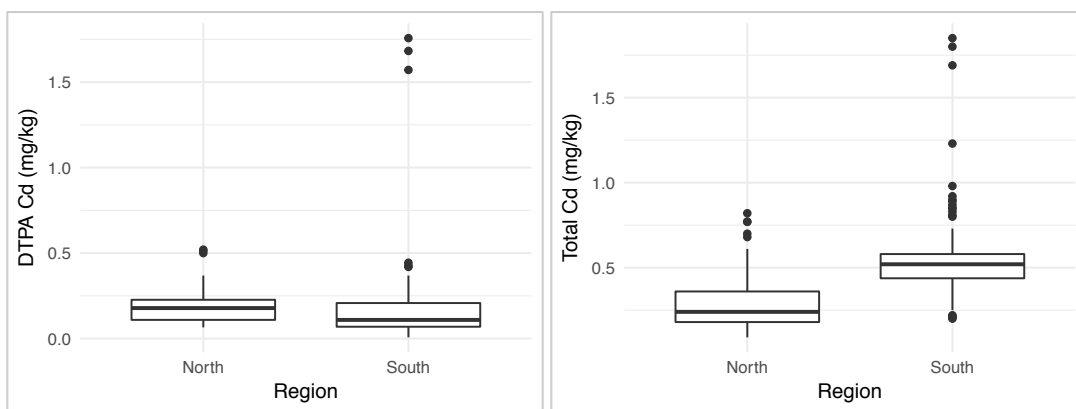
Figure 2.5. (left) Total soil Cd by region (right) DTPA-Cd by region.
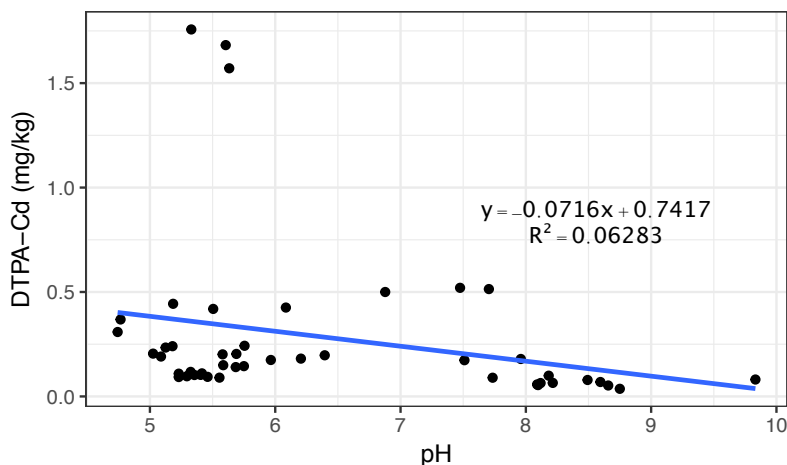
Figure 2.6. Soil pH vs. DTPA-Cd

Less than two percent of the grain sampled was above the FAO's maximum allowable Cd concentration (Figure 2.7). This grain was from Rexburg, Soda Springs, Ashton, and Bonners Ferry, where total soil Cd concentrations were above 0.5 mg/kg. Table 2.3 summarizes grain concentrations of Cd, Cu, Fe, Mn, Zn, and P.

Table 2.3. Grain metal concentrations

	Fe (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	Cd (μg/kg)	P (%)
Mean	40.65	43.07	2.17	30.27	58.68	0.28
Min.	18.70	21.00	0.04	9.10	5.00	0.18
Max.	170.00	108.00	6.82	74.20	510.00	0.40
St. dev.	13.02	11.68	2.01	10.87	45.41	0.04
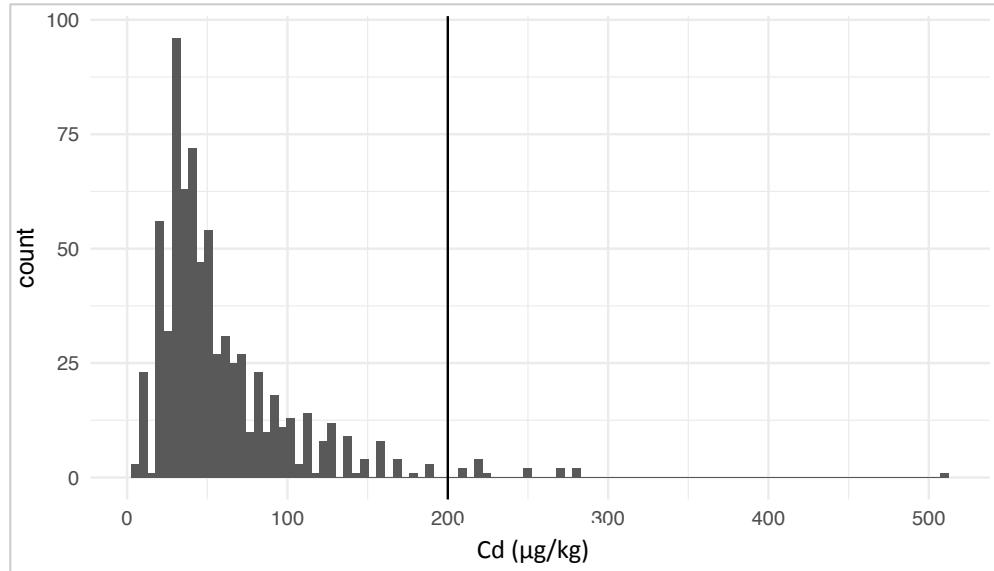
Figure 2.7. Grain Cd concentrations (n = 836). The vertical line represents the FAO's maximum allowable Cd concentration in wheat at 200 μg/kg.

To compare Cd uptake by variety, grain Cd concentration was normalized by soil Cd. Since the experiment was not fully factorial, not all varieties were grown in all soils, and different varieties were therefore exposed to different Cd sources. To account for differences in soil Cd availability, grain concentrations were normalized by location average total soil Cd or DTPA-extractable Cd. Both Cd fractions correlated positively with grain uptake, although neither linear relationship was particularly strong (Figure 2.8). The weak correlation between DTPA soil Cd and grain Cd content found here is corroborated by Ibaraki et al. (2005), who found soil Cd extractable by HCl, NH_4Cl, and $MgCl_2$ correlated better with grain Cd content than DTPA-extractable Cd in a Cd-contaminated soil.
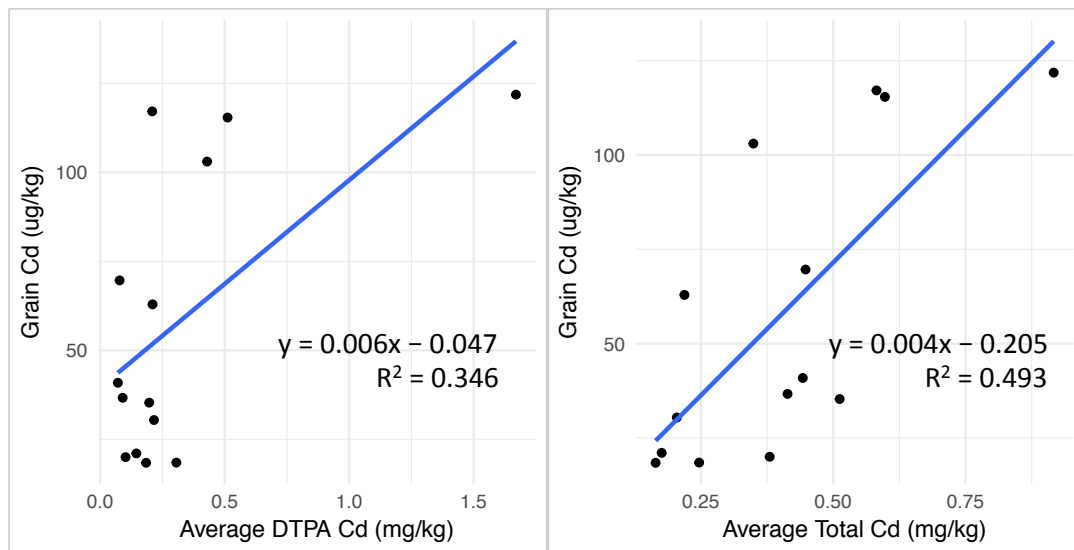
Figure 2.8. Grain Cd concentration vs. DTPA-extractable and total soil Cd. Points represent location averages.

Figure 2.9 compares the distribution of grain Cd, represented by density plots, normalized by both DTPA-extractable and total soil Cd. One-way ANOVA and Tukey's HSD test showed significant ($p < 0.05$) differences between certain varieties. Varieties with significant differences changed between the DTPA and total soil Cd normalized data. When comparing mean grain Cd normalized by total Cd, LCS Star accumulated the most Cd, while UI Silver accumulated the least. Normalized by DTPA-extractable Cd, UI Stone accumulated the most Cd, while UI-WSU Huffman, UI Silver, Norwest 553, and LCS Artdeco accumulated the least. Varieties that do not share a letter are significantly different. Table 2.4 shows classes for the grain varieties in Figure 2.9.

Table 2.4. Variety classes

Hard white spring	UI Platinum, LCS Star
Soft white spring	UI Pettit, UI Stone
Hard white winter	UI Silver
Soft white winter	UI-WSU Huffman, WB Junction, Brundage 96, LCS Artdeco
Hard red winter	Norwest 553
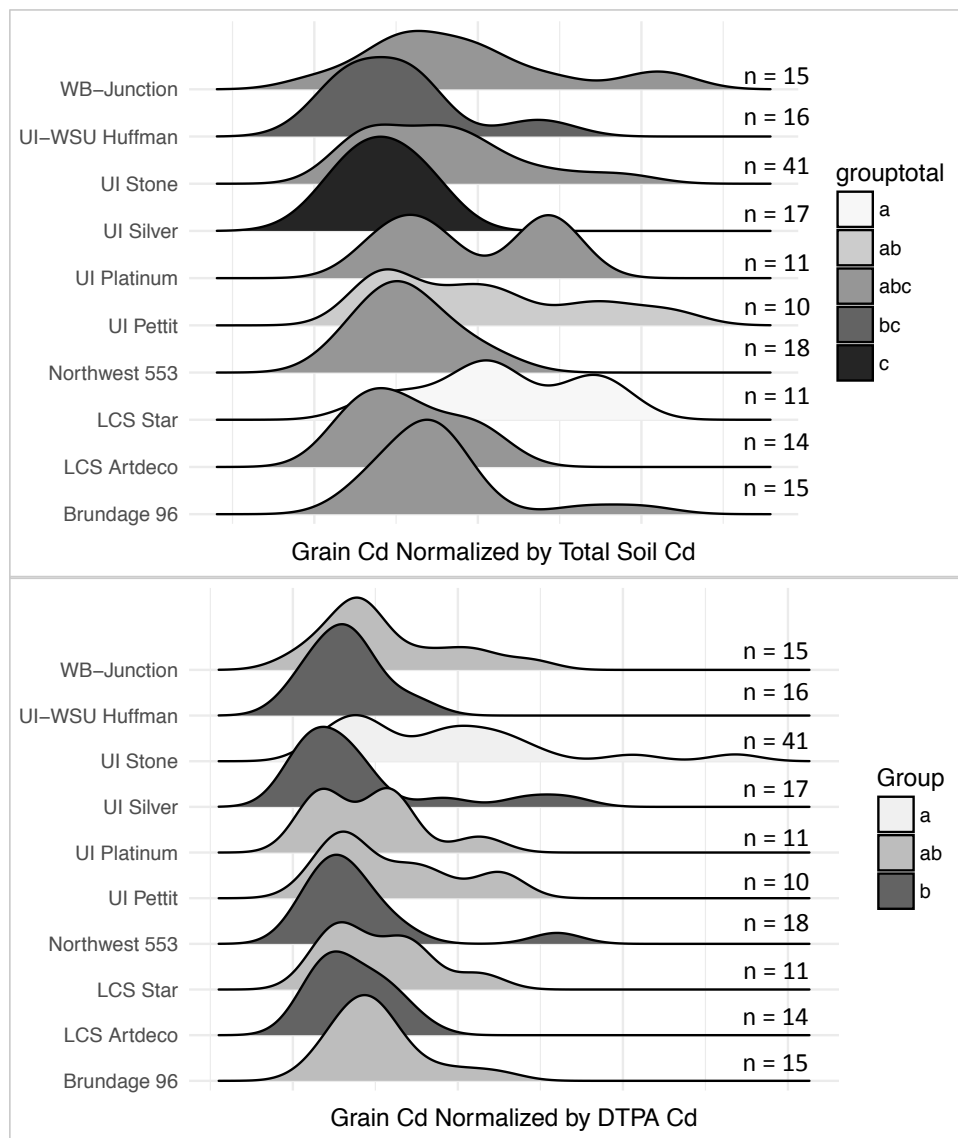
Figure 2.9. Density plot of grain Cd normalized by DTPA-extractable and total soil Cd. Mean and standard deviation for each variety are reported in Appendix Table B.1.

Normalized by both total and DTPA-extractable soil Cd, spring wheat cultivars comprised the groups with the highest Cd uptake, while winter wheat had lower Cd contents. Figure 2.10 shows differences in Cd accumulation by class. The classes with higher Cd accumulation were types of spring wheat. These results are similar to findings by Meyer et al. (1982) that winter wheat accumulated less Cd than spring wheat.
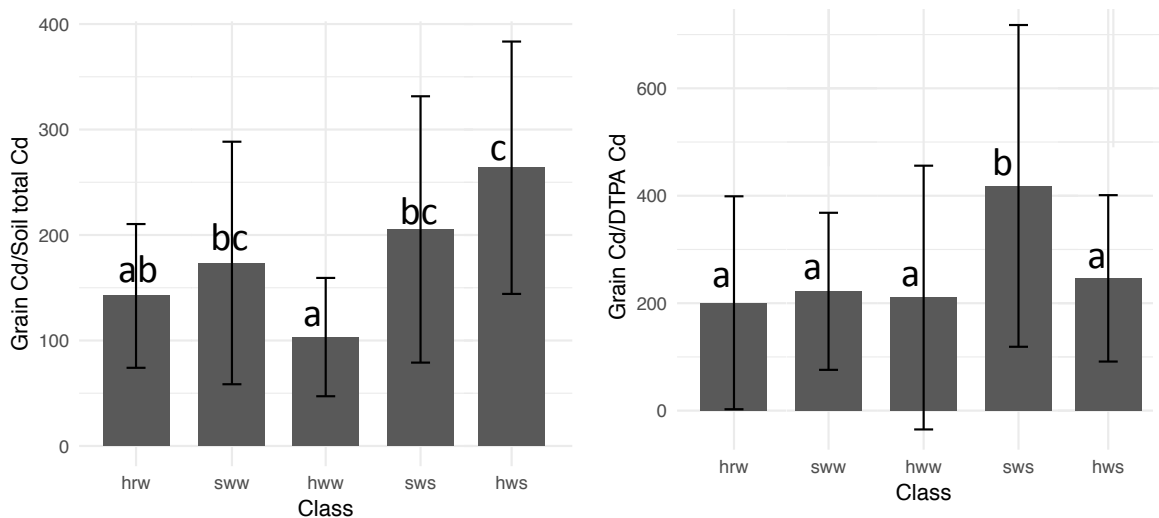
Figure 2.10. Cadmium accumulation normalized by total and DTPA-extractable soil Cd in wheat classes hard red winter ("hrw"), soft white winter ("sww"), hard white winter ("hww"), soft white spring ("sws"), and hard white spring ("hws"). Lines show standard deviation. Significantly different groups ($p < 0.05$) do not share a letter.

Principal Components Analysis

PCA was performed on grain and soil datasets individually, and on the combined data. It was performed on all three datasets separately because combining the grain and soil data required averaging samples by location, resulting in a small sample size that could have obscured relationships within locations. Samples that lacked complete observations for all variables of interest were omitted from this analysis.

In all three datasets, multiple variables were important contributors to PCs. In the soil data, total Cu, DTPA-extractable Mn, total Zn, and total Mn were major contributors to PC1, i.e., they correlated strongly with this component. DTPA-Cu, total Zn, pH, and total Fe were major contributors to PC2. The rest of the correlations between soil properties and PCs 1-6 are shown in Table 2.5.

Table 2.5. Correlations between variables and components in soils. Higher correlations imply a variable contributes more to a PC. Proportion of variance refers to the percent of overall variance that each PC describes. PCs 7-14 are shown in Appendix Table B.2.

	PC1	PC2	PC3	PC4	PC5	PC6
pH	-0.359	0.331	-0.087	0.058	0.011	0.002
MAAT	0.162	-0.127	-0.371	-0.451	0.220	-0.076
MAP	0.291	-0.284	-0.151	-0.240	-0.245	0.287
DTPA Cd	-0.004	-0.148	0.557	-0.040	0.127	0.261
DTPA Cu	-0.186	0.335	0.078	-0.445	-0.040	-0.417
DTPA Fe	0.291	-0.177	0.223	0.338	0.069	-0.448
DTPA Mn	0.363	0.019	0.221	0.237	0.227	-0.286
DTPA Zn	0.042	0.051	0.428	-0.514	-0.002	-0.295
Total Cd	-0.221	-0.161	0.447	-0.161	0.098	0.396
Total Zn	0.347	0.347	0.067	-0.045	-0.195	0.164
Total Cu	0.381	0.298	0.054	-0.097	-0.143	0.089
Total Mn	0.348	0.315	0.062	-0.011	-0.231	0.214
Total P	-0.160	-0.290	0.100	0.043	-0.828	-0.237
Total F	0.218	-0.454	-0.112	-0.250	0.103	-0.076
Standard deviation	2.164	1.705	1.636	1.175	0.875	0.820
Proportion of variance	0.335	0.208	0.191	0.099	0.055	0.048
Cumulative proportion	0.335	0.542	0.733	0.832	0.887	0.935

In the grain dataset, Zn, P, MAP, and MAAT contributed significantly to the variation expressed by PC1, and Cd and Cu were important in PC2 (Table 2.6).

Table 2.6. Correlations between variables and components in grain.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Fe	0.033	-0.346	0.476	-0.363	0.699	-0.023	0.178	-0.025
Mn	-0.135	-0.301	0.369	0.698	0.001	-0.483	-0.187	0.008
Cu	-0.098	-0.633	-0.462	0.023	0.134	0.252	-0.370	0.396
Zn	-0.467	-0.336	-0.334	0.067	-0.053	-0.057	0.602	-0.430
Cd	-0.023	-0.406	0.448	-0.327	-0.694	0.089	0.104	0.162
P	-0.551	0.075	0.161	-0.202	-0.009	0.164	-0.604	-0.482
MAAT	0.491	-0.217	0.112	0.347	-0.022	0.581	-0.013	-0.490
MAP	0.455	-0.240	-0.271	-0.328	-0.098	-0.571	-0.243	-0.399
St. dev.	1.506	1.177	1.136	1.043	0.886	0.701	-0.621	0.554
Proportion of variance	0.283	0.173	0.161	0.136	0.098	0.061	0.048	0.038
Cumulative proportion	0.283	0.456	0.618	0.754	0.852	0.913	0.962	1.000

In the combined dataset, grain P, grain Zn, and total soil Cd correlated relatively well with PC1, while DTPA-Cd, DTPA-Fe, and total soil P correlated with PC2 (Table 2.7).

Table 2.7. Correlations between PCs and variables in the combined soil-grain dataset. The rest of the PCs are shown in Appendix Table B.3.

	PC1	PC2	PC3	PC4	PC5	PC6
Grain Cd	0.221	0.236	0.198	0.364	-0.064	-0.171
Grain Cu	-0.212	-0.194	-0.100	0.376	-0.139	-0.101
Grain Fe	-0.237	-0.122	0.207	0.154	-0.409	0.029
Grain Mn	-0.080	0.110	0.100	-0.358	-0.410	-0.555
Grain P	0.337	-0.060	0.073	-0.295	0.115	-0.106
Grain Zn	0.319	-0.055	-0.166	-0.150	-0.142	-0.278
DTPA Cd	0.131	0.424	0.109	0.187	-0.112	0.327
DTPA Cu	0.202	0.001	-0.446	-0.168	-0.044	0.208
DTPA Fe	-0.203	0.314	0.237	-0.171	0.070	0.237
DTPA Mn	-0.240	0.255	0.112	-0.262	-0.315	0.128
DTPA Zn	0.222	0.225	-0.282	-0.154	-0.327	0.062
Total soil Cd	0.301	0.186	-0.004	0.279	-0.171	0.048
Total soil Cu	-0.174	0.129	-0.477	-0.082	0.023	0.288
Total soil Fe	-0.327	0.270	-0.142	0.069	0.045	-0.135
Total soil Mn	-0.221	0.254	-0.149	-0.209	0.096	-0.130
Total soil P	0.153	0.278	0.005	0.009	0.484	-0.255
Total soil Zn	0.081	0.228	-0.338	0.352	-0.205	-0.218
MAP	-0.269	0.260	-0.112	0.124	0.256	-0.311
MAAT	-0.209	-0.306	-0.332	0.087	0.002	-0.093
Standard deviation	2.492	1.788	1.668	1.462	1.269	1.024
Proportion of variance	0.327	0.168	0.146	0.113	0.085	0.055
Cumulative proportion	0.327	0.495	0.642	0.754	0.839	0.894

Figure 2.11 graphically represents correlations between variables and PCs 1 and 2. In the grain dataset, Cd concentration was slightly correlated with Fe and Cu, but unrelated to climate or other metals. The weak relationship between grain Cd and Zn contradicts the common observation in the literature that the two compete for uptake. Similarly, in the combined dataset, DTPA Zn was positively correlated with grain Cd, along with soil Cd and total soil P. MAAT was unrelated to Cd availability in soil, but inversely related to grain Cd in the combined dataset. Soil P and total soil Cd were correlated in the soil and combined datasets. However, grain P concentrations were unrelated to grain Cd concentrations.

In PCA, each variable is assigned a coefficient, or a loading, that reflects its contribution to the PCs. Multiplying the loadings and the original measured values yields scores, i.e. the transformed values for each data point. Figure 2.11 shows scores for Northern

and Southern Idaho grain and soil for PCs 1 and 2. Northern Idaho grain is slightly higher than Northern Idaho grain along the PC1 axis. PC1 describes MAP and MAAT, which were positively correlated with the component, and P and Zn, with which the component was negatively correlated. Northern soils show more variation along the PC1 axis, which primarily describes total Cu, DTPA-extractable Mn, total Zn, and total Mn. Southern soils show more variation for PC2, which primarily describes DTPA-Cd, pH, and total Fe. In the combined dataset, Southern Idaho grain is higher along PC1, which primarily describes grain P, grain Zn, and total soil Cd.

PCA revealed potentially important relationships among grain and soil nutrients. In the grain, Cd and Zn did not demonstrate antagonism for uptake or availability in the soil, despite numerous accounts of competition in the literature (Hart et al., 2002). Since antagonism is most often observed in Zn-deficient soils, it may have been absent in these data because many of the soils contained sufficient Zn for wheat growth (Chaney et al., 2006). Less than 2 percent of the soils in this data were below the DTPA-extractable Zn deficiency threshold of 0.4-0.6 mg/kg (Mahler et al., 1981). In the soil, positive correlation between total P and Cd could indicate frequent Cd contamination in P fertilizer. Grain Cd accumulation was positively correlated with total soil P, which could indicate P contamination with Cd or other mechanisms of P-induced Cd uptake. Cadmium accumulation may also be related to climate, either through the effects of moisture and temperature on nutrient availability and plant growth or geographic differences that simultaneously influence climate and other soil forming factors. In these data, Cd uptake appeared to be greater in colder climates. Further research should explore the conditions under which Cd and Zn compete, the influence of soil P status on Cd availability, and the effects of climate on Cd accumulation.
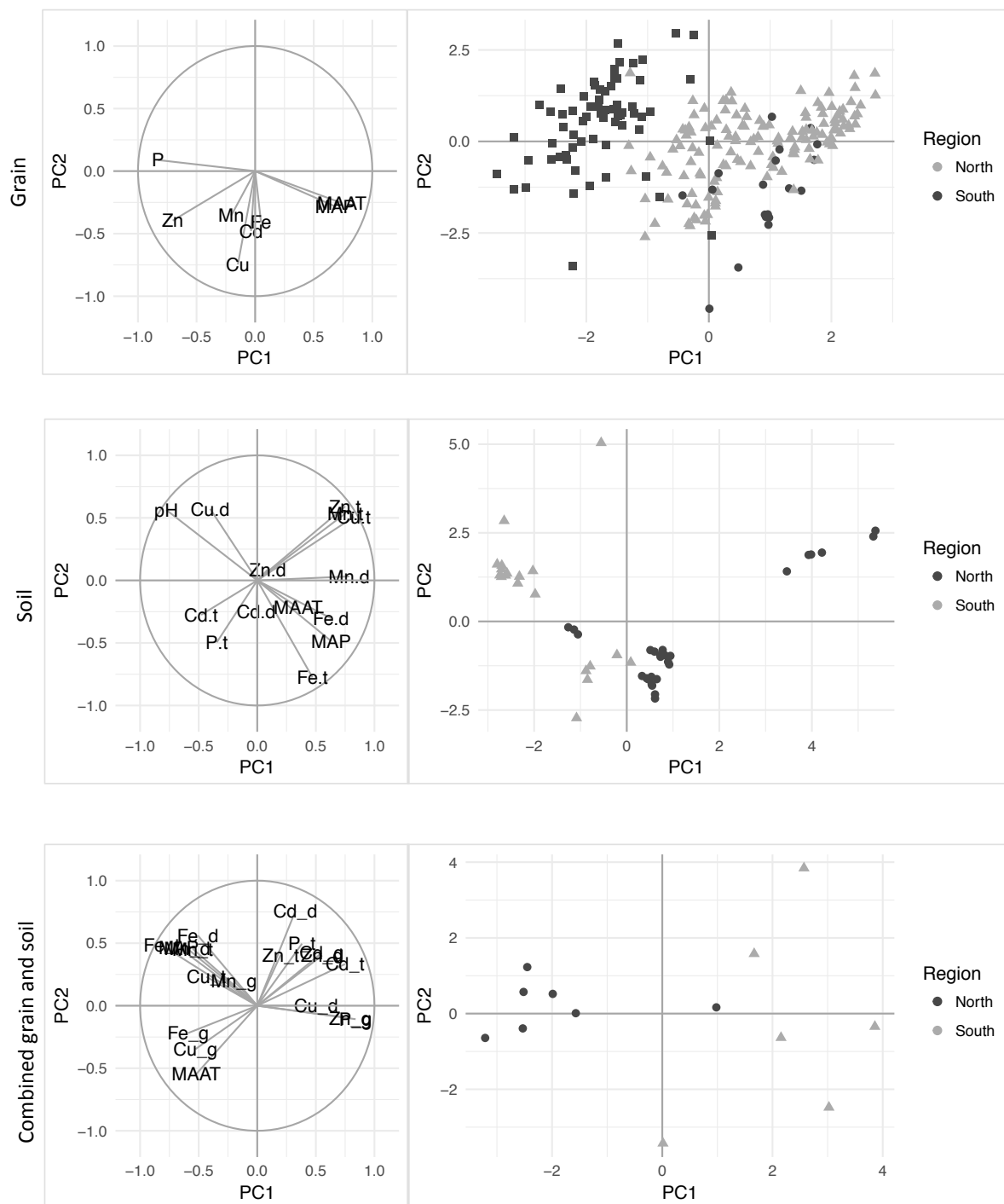
Figure 2.11. Left: correlations between variables and PCs. Longer lines indicate greater variation. The closer lines are to being parallel, the more correlated they are. "_g" refers to grain concentrations, "_t" to total soil metals and "_d" to DTPA-extractable soil metals. Right: Scores for observations by region.

Relationships Between Grain Cd and Soil and Grain Properties

As described in Chapter 1, Cd accumulation in wheat grain varies based on the availability of soil Cd and other trace metals and nutrients. Previous research has observed relationships between soil properties and Cd uptake in grain, such as a negative relationship between soil pH and grain Cd and between grain Zn and grain Cd (Baize et al., 2009). Relationships between Cd accumulation and grain, soil, and climate properties were assessed using Pearson correlations (Table 2.8).

Table 2.8. Spearman correlations between grain Cd concentration and grain and soil properties. ** $p < 0.01$ * $p < 0.05$

	Independent variable	**Correlation with grain Cd**
Grain	Cu	0.050
	Fe**	0.118
	Mn	0.041
	P**	0.329
	Zn**	0.223
Soil	pH	0.142
	EC	-0.169
	Cd_{DTPA}**	0.588
	Cu_{DTPA}	-0.070
	Fe_{DTPA}	-0.083
	Mn_{DTPA}	-0.264
	Zn_{DTPA}	0.103
	Cd_{Total} **	0.702
	Zn_{Total}	0.453
	Cu_{Total}*	-0.502
	Mn_{Total}	-0.366
	P_{Total}	0.338
	Fe_{Total}	-0.183
Climate	Mean annual precipitation	-0.286
	Mean annual air temperature*	-0.535

These correlations differ from those obtained using PCA because they use the complete grain and soil datasets, while the PCA data omitted grain samples that did not include measurements for all variables of interest. Grain P, Fe, and Zn concentration showed significant positive correlations with grain Cd, despite the tendency in the literature for Zn and Cd to compete for uptake (Hart et al., 2002). Zn-Cd synergism is occasionally observed in the literature, such as when Zn concentrations are so high that they damage tissues and permit

nonselective uptake of Cd (Sarwar et al., 2010). However, toxic concentrations of Zn are rare in Idaho soils: less than 2 percent of soils in this data contained above 7 mg/kg DTPA-extractable Zn, which Takkar and Mann (1977) observed to be the threshold of toxicity for wheat. Antagonism is observed primarily in Zn-deficient soils (Chaney et al., 2006), which were rare in these data. Future research should clarify under what conditions Zn-Cd antagonism and synergism occur to inform Cd management strategies like Zn supplementation.

Although many researchers have found Cd uptake and soil pH are inversely related (Baize et al., 2009), there was no significant relationship between the two in these data. This may be due to confounding factors such as organic matter content, which was not measured in this project. Total soil Cu appeared to be antagonistic with grain Cd. This may simply be because DTPA-Cu was positively correlated with pH. However, this antagonism is congruent with findings by Murtaza et al. (2017) that applying a combination of Cu and Zn to soils spiked with Cd decreased grain Cd accumulation, although Cu applied alone did not significantly affect Cd uptake. Koleli et al. (2004) found that Cu and Zn competed for uptake. These results suggest Cu may affect Cd uptake through its relationship with Zn. Researchers may experiment with Cu supplementation and investigate varieties that exhibit competition between Cu and Cd, which appeared to be slightly antagonistic here.

Competitive uptake dynamics may differ by variety. Figure 2.12 shows relationships between metals (excluding Fe, which was not measured in many samples) in different varieties to assess interactions between variety and metal concentrations. Only varieties that exhibited significant ($p < 0.05$) correlations between metals are shown. R^2 values are shown in Table 2.9.
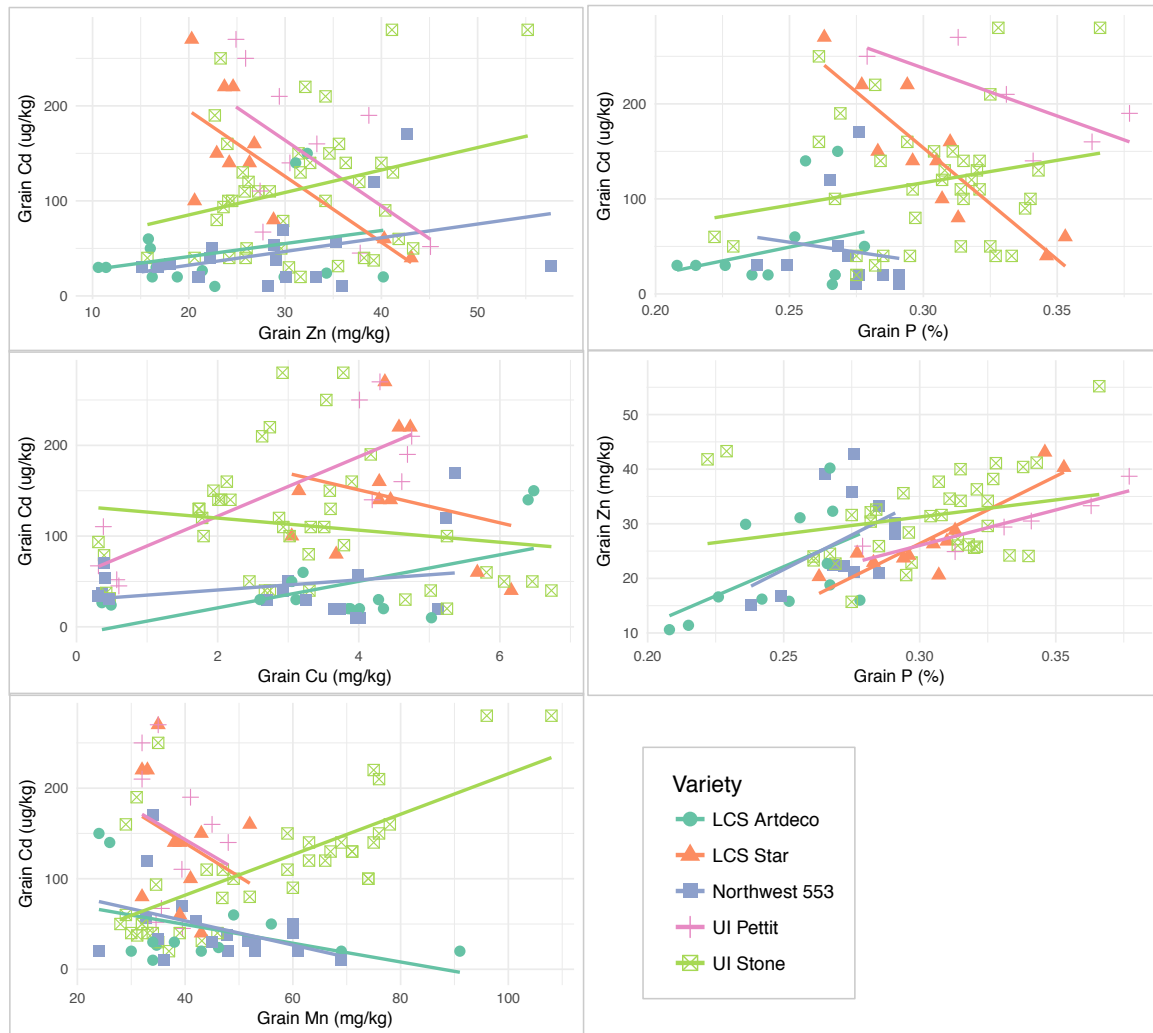
Figure 2.12. Relationships in grain by variety

Table 2.9. R^2 values for linear correlations between grain metals and grain Cd. * $p < 0.05$

	LCS Artdeco	LCS Star	Norwest 553	UI Pettit	UI Stone
Zn	0.083	**0.525***	**0.358***	0.314	0.073
Mn	0.179	0.094	0.169	0.054	**0.450***
Cu	**0.347***	0.056	**0.344***	**0.695***	0.025
P	0.079	**0.781***	0.018	0.495	0.045

Element-to-element relationships varied considerably among varieties, with both positive and negative correlations. For example, LCS Star exhibited antagonism between Zn and Cd, while Norwest 553 showed synergism. UI Stone showed strong positive correlation between Cd and Mn uptake, while the other varieties showed weak antagonism. Cu uptake showed positive correlation with Cd in LCS Artdeco, Norwest 553, and UI Pettit. The

relationship between a variety's class and whether it exhibited synergisms or antagonisms was unclear: only spring wheat varieties showed Cd-Zn and Cd-Cu antagonism, but other mineral relationships occurred in a mix of classes.

The positive relationship between P and Zn in all varieties appears counterintuitive, considering the evidence in the literature that higher P application rates limit Zn availability. However, higher P application can be associated with lower P concentration in grain due to dilution from increased growth (Akhtar et al., 2016). In this case, P application would be linked to Zn suppression. The strong negative correlation in LCS Star between P and Cd may therefore mean that increased P application was associated with increased Cd uptake. Judgments about the influence of variety are tentative here due to the small number of observations and the fact that the data were not collected from a fully factorial experimental design.

The effects of location may confound the influence of variety. The dataset used in this analysis lacked potentially important environmental characteristics such as organic matter content. Additionally, not all varieties were grown in all locations, and some locations were dominated by one variety. For example, UI Stone grew primarily in Rexburg, UI Silver in northern Idaho, and LCS Star in southern Idaho. The relationships they exhibited may have had more to do with location attributes than physiological ones. However, only UI Stone was dominated by one location, and all varieties were grown in at least 5 locations (Appendix Table B.4 shows locations for varieties). Nutrient relationships in grain persisted across locations. Provided these locations had sufficiently varied environmental characteristics, this suggests that differences in mineral relationships between varieties were due to variety, not environmental factors. If different varieties growing in the same location showed different relationships between metals, that would provide further evidence for the influence of variety on metal accumulation; however, these sample sizes were too small to reliable assess trends within locations. These data suggest that cultivars LCS Artdeco, LCS Star, Norwest 553, UI Pettit, and UI Stone may exhibit unique uptake dynamics between Cd and other metals. Learning more about their physiology could help researchers select genetic traits to control Cd uptake. Future research should explore the influence of these varieties on Cd accumulation.

Conclusion

DTPA-extractable and total soil Cd and grain variety appeared to be important determinants of Cd accumulation in wheat. DTPA-Cd's correlation with Cd uptake is unsurprising; however, we did not observe other expected relationships between soil properties and grain Cd accumulation. Although the sampling strategy's limitations may have confounded the analysis, these results caution against assuming expected relationships between trace metals, particularly Cd-Zn antagonism. The assumption that Zn and Cd compete for uptake may not be valid in regional soils, particularly because competition has mostly been observed in Zn-deficient soils, which have a limited range in Idaho (Mahler et al., 1981). Future studies should clarify under what conditions Zn-Cd antagonism occurs to inform Cd management strategies, like Zn supplementation.

Grain variety appeared to influence both Cd accumulation and its relationships with other trace metals. UI Silver, UI-WSU Huffman, LCS Artdeco, and Norwest 553 showed evidence of limiting Cd uptake, while UI Stone appeared to absorb more. Cd-Zn antagonism was only observed in UI Platinum, while other varieties exhibited a variety of antagonisms and synergisms between elements. Grain P concentration—which may be inversely related to P fertilizer application, due to growth-driven dilution—correlated positively with Zn in most varieties and negatively with Cd in some. These results point toward a need for more research regarding the influence of variety on the relationship between Cd and other trace metals in plant uptake. Variety selection, and supplementation with nutrients specific to uptake dynamics exhibited by each variety, could become effective Cd management tools.

Chapter Three: Effects of Andic Mineralogy on Aluminum Toxicity in Acidifying Soils

Introduction

Soil acidification is a pervasive issue in Northern Idaho that can lower wheat yields (Koenig et al., 2011). Long-term nitrogen fertilization can acidify soil through nitrification. North Idaho soils are particularly susceptible to acidification, since high precipitation leaches buffering minerals like calcium carbonate. Before cultivation, prairie soils of the Palouse region were typically near neutral pH; it is now common to find soils with pH below 5 (McFarland et al., 2015). The primary risk to crops associated with soil acidification is aluminum (Al) toxicity. As pH decreases, soluble Al increases to potentially phytotoxic levels, causing root stunting and reduced yields (Koenig et al., 2011).

Mineralogical differences among Palouse soils may have implications for Al toxicity under acidification. Regional agriculture uses soil that developed under both grassland and forest. While phyllosilicates dominate the clay fraction of prairie soils, ash-influenced forest soils contain a greater portion of poorly crystalline minerals like allophane and imogolite and organically complexed Al. The US Department of Agriculture defines soil properties associated with volcanic ash, or andic properties, as a combination of low bulk density, high amorphous mineral content, high volcanic glass content, and high phosphorus retention (Soil Survey Staff, 2014). Additionally, andic soils have higher NaF pH and more organically complexed Al than ash-free soils. Since andic soils in the region developed under conifer forest, their pH tends to be lower than that of uncultivated prairie soils (Fisher and Binkley, 2013). Some forest soils converted to agriculture may retain andic properties due to volcanic ash influence, although tillage mixes ash throughout the profile and cultivation can weaken andic properties due to increased aeration and moisture and temperature fluctuations (Verde et al., 2010).

The unique speciation of Al in ash-influenced soils may lead to different Al availability under acidification, although it is unclear whether Al toxicity is more or less severe in Andic soils than non-Andic ones. The relationship between amorphous Al, organic matter, pH, and Al availability is complex. Andic mineralogy has the potential to mitigate Al

toxicity at low pH because of strong organo-mineral complexation. Soil organic matter content has been observed to decrease the activity of Al^{3+} and alleviate Al toxicity by raising soil pH and forming stable complexes with Al (Vieira et al., 2008). The most toxic form of soil Al is thought to be Al^{3+}, followed by hydrolysis species $AlOH^{2+}$, $Al(OH)_2^+$, and $Al(OH)_4^-$ (Bojórquez-Quintal et al., 2017). Haynes and Molokobate (2001) write that organic matter forms complexes with Al that make it unavailable to plants, citing studies that found addition of organic residues decreased monomeric Al concentrations. Organically complexed Al is commonly considered less toxic than Al^{3+} at similar pH (Brown et al., 2008). Andic soils contain an unusually high proportion of organic matter in the form of Al-humus complexes. Poorly crystalline minerals common in Andisols physically occlude soil organic matter and prevent its degradation through toxicity to microbes, increasing the residence time of organic C in soil and resulting in high organic matter content (Buurman et al., 2007) Volcanic minerals like ferrihydrite have high specific surface area and abundant reactive sites that undergo ligand exchange and electrostatic bonding with organic matter functional groups. Negatively charged functional groups on organic matter can enter the tiny spherules and pores that comprise volcanic minerals like allophane and imogolite and form strong bonds with OH-groups on their surfaces (Filimonova et al., 2016). The stability of Al complexation with organic matter in andic soils could alleviate Al toxicity at low pH.

Crystalline Al forms like gibbsite are thought to control Al availability in most soils. The solubility curve for gibbsite, which describes equilibrium concentration of soluble Al in relation to pH, has a slope of about three (Figure 3.1) (Takahashi et al., 2003). However, in soils with low permanent charge cation exchange capacity relative to organic matter, Al availability is controlled by Al-humus complexes, which have slopes less than three (Figure 3.1). Takahashi and Dahlgren (2016) found that soils with abundant Al-humus complexes have pAl/pH slopes between 1.3 and 2.4. Brown et al. (2008) found that below pH 5.5, Al^{3+} was undersaturated with respect to gibbsite and the slope of the Al^{3+} solubility curve remained flat with decreasing pH, indicating that soil organic matter buffered (Al^{3+}) at low pH. Since organically bound Al produces a solubility curve with a lower slope than crystalline Al species such as gibbsite, these soils might buffer Al better than soils without Andic properties at low pH. However, Brown et al. (2008) found that Mollisols typical of the region had enough organic matter to exhibit the effect independent of poorly crystalline minerals.
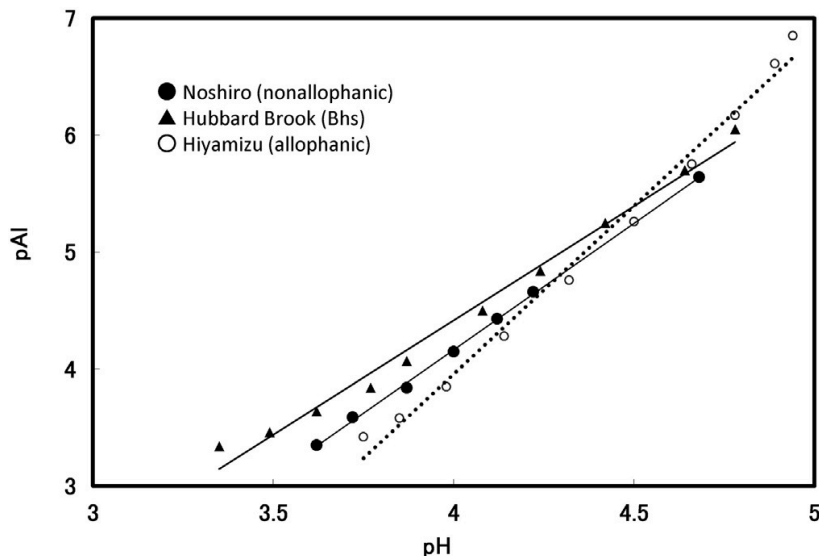
Figure 3.1. From Takahashi and Dahlgren (2016): "Equilibrium Al solubility vs. pH 25 °C for A horizons of non-allophanic and allophanic Andosols and a Bhs horizon of a Podzol. The solubility of synthetic gibbsite is indicated by the dotted line for comparison."

Amorphous Al minerals may mitigate acidification as well. Camps-Arbestain et al. (2003) found soils with a greater proportion of poorly crystalline minerals were better able to buffer acidity from weak acids associated with organic matter. They suggest that the abundance of reactive Al surfaces stabilized organic acids, maintaining higher pH and resulting in lower soluble Al. Thus, although Al saturation appears to determine the capacity of organic matter to sorb metals, their study found the more amorphous mineral-rich, Al-saturated soil actually had lower Al^{3+} concentrations.

There is also evidence that andic soils may exhibit more severe Al toxicity at low pH. Several studies have found organically bound Al, which is more abundant in andic soils, can be a source of plant-available Al^{3+}, despite research suggesting organic complexation ameliorates Al toxicity. Takahashi et al. (2011) found KCl-extractable Al correlated well with the pyrophosphate-extractable fraction, which is relatively high in andic soils. Since Al extractable by KCl is typically thought to approximate the plant-available fraction, these results suggest that a considerable portion of organically bound Al contributes to toxicity. Mora et al. (2012) found the Al species best correlated to bioaccumulation in plants was Al extracted by EDTA. Although the study did not directly measure uptake of organically bound Al, EDTA is a chelating agent that can extract metals from Al-humus complexes, indicating organic Al was an important source of plant-available Al.

Non-crystalline Al exhibits faster dissolution kinetics than crystalline forms. If the residence time of soil water is too short for crystalline Al to reach equilibrium, but long enough for non-crystalline forms, the latter could contribute more Al to the solution (Takahashi et al., 1995). Dahlgren et al. (1994) found that in both allophanic and non-allophanic Andisols, Al release rates declined substantially after pyrophosphate treatment but little after KCl and ammonium oxalate treatments, indicating that organic complexes were a major source of dissolved Al. Aluminum saturation of organic matter may also make organically bound Al a source of toxicity. The degree of Al saturation of carboxylic groups on humic substances determines the slope and intercept of the relationship between pH and Al^{3+} activity (Cronan et al., 1986). Cronan et al. (1986) found that slope increases with bound aluminum ratio, i.e. the molar ratio of organically bound aluminum to total carboxyl functional groups. In their study, as the ratio of pyrophosphate-extractable Al to organic C increased, the pAl/pH line approached that of gibbsite. If, as Takahashi et al. (2010) suggest, Al_p correlates with Al-saturation, soils with more organically bound Al will exhibit a steeper, more gibbsite-like solubility curve. Since andic soils tend to have greater pyrophosphate-extractable Al, they could exhibit Al toxicity that is equally severe as that of non-andic soils under acidification.

The capacity of andic soils to buffer or exacerbate Al toxicity at low pH may vary based on other factors, such as allophanic or non-allophanic mineralogy and organic matter quality. Most Al in allophanic Andisols is in the form of short-range order minerals, while non-allophanic Andisols are dominated by Al-humus complexes and tend to have higher KCl-extractable Al and lower pH. Given the differences listed above between poorly crystalline and organically bound Al, the allophanic distinction could affect andic soils' Al buffering capacity. Ito et al. (2009) grew barley in perlite mixed with synthetic Al-humus complexes derived from allophanic and non-allophanic Andisols and found that both decreased root growth; however, adding synthetic allophane to the perlite-Al-humus mixture ameliorated Al toxicity and eliminated Al ions in solution, suggesting allophanic mineralogy can reduce Al toxicity. However, Yamada et al. (2011) found allophanic mineralogy did not inhibit Al toxicity at low pH relative to non-allophanic soils. Acidification can change an allophanic soil into a non-allophanic one: Takahashi et al. (2008) found that allophanic Andisols acidified due to nitrogen fertilization showed a decrease in allophanic materials and an increase in Al-

humus complexes. Organic matter also varies in its capacity to mitigate or exacerbate Al toxicity. Eimil-Fraga et al. (2015) found that more evolved organic matter—in other words, organic matter with low C/N ratios—was associated with greater quantities of oxalate- and pyrophosphate-extractable Al, while soils with higher C/N ratios contained more exchangeable and labile Al. Variability within andic soils such as allophanic status and organic matter quality complicate the effects of andic mineralogy on Al availability.

Research Goal

Clarifying the impact of andic soil properties has practical importance for Northern Idaho agriculture. If ash mineralogy changes Al availability at low pH, the critical pH at which Al becomes phytotoxic may be different from the ash-free prairie soils more typical of Palouse agriculture. A better understanding of these processes will inform growers' soil pH management.

This experiment tested the hypothesis that Al availability under decreasing pH would differ significantly between soils with ash influence and those without ash. For the sake of simplicity, the traditional definition of Al availability as the quantity of Al bound to exchange sites extractable by 1 M KCl was used, although both gentler extractants (e.g., $CaCl_2$) and extractants associated with organic matter (e.g. $CuCl_2$) have been observed to correlate better with Al bioaccumulation (Bertsch and Bloom, 1996). According to this hypothesis, available Al varies according to some combination of pH, ammonium oxalate-extractable metals, pyrophosphate-extractable metals, organic matter content and quality, NaF pH, and glass content. These claims were assessed in two ways. First, we assumed andic mineralogy was a binary variable—volcanic ash influence was either present or absent—and determined whether there was an interaction between mineralogy and pH with respect to Al availability. However, since andic properties exist in soils on a continuum, we also assessed the influence on Al availability of andic indicators as continuous variables, modeling plant-available Al as a function of these properties. The fact that cultivation weakens andic properties with time made accounting for intergrades between ash-heavy and ash-free soils especially important (Verde et al., 2010). The presence of an interaction would indicate that the risk of Al toxicity with decreasing pH differs between ash-influenced and ash-free soils. Modeling Al availability on measured soil properties will help determine which properties of ash-influenced soils affect Al availability.

Methods

Sampling

Sampling sites were chosen from a database of locations in North Idaho with measurements of nutrient availability, pH, EC, OM, soil classification, texture, cropping system, and productivity. The sites were on land under pasture, ungrazed grasses, or wheat production. Pairs of soils—with and without ash influence—were selected along a pH gradient of 4.5 to 6.5, with organic matter content restricted to between 3 and 4 percent. The presence of volcanic ash was assumed based on the NRCS mapped classification of the sites. After an initial screening of the surface soils for poorly crystalline minerals using NaF pH, we found few of the sites mapped as Vitrandic met the NRCS threshold. This is unsurprising, given that the scale of soil mapping necessitates variability within map units, particularly units comprised of complexes. Several more sites thought to have andic properties were included to ensure there were enough soils with ash influence. In total, 15 sites were sampled—6 mapped as non-Vitrandic, 9 mapped as Vitrandic.

At each site, 3 subsamples were collected from the top 6 inches within a 100-foot radius of the mapped point. The subsamples were taken from different landscape positions to capture variation within the site. Subsamples were homogenized and analyzed as composites.

Chemical analyses

Soil pH was measured in a 1:1 paste of soil and triple-distilled water. KCl-extractable Al (Al_{KCl}) was measured using a 1:5 solid-solution ratio of 1 M KCl. Samples were shaken for 5 minutes, centrifuged, filtered through 0.45 μm polyether sulfone (PES) membrane filters, and diluted to a 1:5 ratio for analysis on an ICP-AES (Thermo Electron, Waltham, MA) (Thomas, 1996). NaF pH was measured using a 1:50 solid-solution ratio in 1 M NaF (Soil Survey Staff, 2014). Total N and C were determined via dry combustion using a Vario Max CNS analyzer (Elementar Americas, Inc., Mt Laurel, NJ). Ammonium oxalate-extractable Al (Al_o) was obtained using a 1:100 solid-solution ratio in 0.11 M $C_2H_8N_2O_4$ and 0.09 M $C_2H_2O_4$ (Soil Survey Staff, 2014). Samples were shaken in the dark for 4 hours, centrifuged, filtered through 0.45 μm PES filters, and diluted to a 1:5 ratio (Bertsch and Bloom, 1996). Pyrophosphate-extractable Al (Al_p) was obtained with a 1:60 solid-solution ratio in 0.1 M $Na_4P_2O_7$. Samples were shaken overnight, centrifuged with 10 drops Superfloc,

filtered through 0.45 μm PES filters, and diluted to a 1:10 ratio (Soil Survey Staff, 2014). Glass counts were performed on the very fine sand fraction (0.05-0.1 mm). Sieved samples were treated with sodium citrate, bicarbonate, and sodium dithionite over heat to remove iron staining. Samples were set in oil with refractive index n = 1.5150, and glass was counted on a polarized microscope.

All procedures were done in triplicate except N and C analysis and glass counts, which were performed on single samples. Al_o and Al_p extractions were performed on reference samples (recoveries are listed in Appendix Table 3.7).

Statistical analyses

The influence of andic mineralogy was investigated using two analyses: 1) checking for statistical interaction between andic status and pH as predictors of Al availability, and 2) modeling Al availability on soil andic properties. For the first analysis, we assumed andic mineralogy was a binary variable—volcanic ash influence was either present or absent—based on the amount of sand-sized particles, volcanic glass, and poorly crystalline minerals (the same criteria USDA soil taxonomy uses to distinguish the Vitrandic subgroup). The purpose of classifying the soils this way was to create a simple, binary test of whether a soil had significant ash influence, not to determine whether the soil met full taxonomic requirements. These requirements apply to entire pedons with specified depths, whereas this experiment focused on the top 6 inches of the profile, which are most important to seedling growth. This analysis was intended to assess whether ash influence changed the effect of pH on Al_{KCl}—in other words, if an interaction between andic status and pH occurred. We modeled Al_{KCl} on andic status and pH, including an interaction term between the two. Andic status was coded as a dummy variable in the regression model (Fox, 2015).

Properties associated with volcanic ash vary by degrees rather than as a binary. We therefore also assessed the influence on Al availability of andic properties as continuous variables. Since the sample size was small, the number of predictors was limited for parsimony. Additionally, many of the properties tested were collinear, limiting the number of model predictors. Predictors that correlated well individually with Al_{KCl} were initially chosen, then eliminated to those that produced the best model. Models were evaluated based on mean squared error, variance inflation factor of predictors, adjusted R^2, and goodness of fit

according to the corrected Aikake Information Criterion (Hurvich, 2003). Since organically bound Al can also be a source of Al toxicity, Al_p was modeled in the same way.

Results

Table 3.1 shows pH, N, C, C/N ratio, NaF pH, oxalate-extractable Al, Fe, Mn, and Si, pyrophosphate-extractable Al, Fe, and Mn, volcanic glass, and Al_p/C ratio. Al_p/C ratio is used to represent Al saturation of organic matter (Camps-Arbestain et al., 2003). In some cases, recovery rates for oxalate- and pyrophosphate-extractable metals were less than 85 percent of reference values (Appendix Table C.1). Table 3.2 shows USDA mapped classification of the sites to the subgroup and series level and current land use. The sites in the category "significant ash influence" met the chemical requirements applied to the vitrandic subgroup. According to USDA soil taxonomy, soils in this subgroup must 1) be comprised of at least 30 percent sand-sized particles, 2) have at least 5 percent volcanic glass in the sand fraction, and 3) must satisfy the equation $60 * (Al_o + \frac{1}{2} Fe_o) + glass > 30$ (Soil Survey Staff, 2014). For the purposes of this experiment, the threshold is used to denote soils with significant ash influence rather than verify USDA mapping and classification.

Table 3.1. Soil properties. One homogenized sample represented each site. Most properties were measured in triplicate, except total N, C, and glass, which were measured with 1 replicate. Means and standard deviations of lab replicates are provided here.

	Site	Mean SD	pH	N (%)	C (%)	C:N	NaF pH	Al_{KCl} (cmol$_c$/kg)	Al_o (mg/kg)	Fe_o (%)	Mn_o (%)	Si_o (%)	Al_p (%)	Fe_p (%)	Mn_p (%)	Glass (%)	Al_p/C
	1	mean	5.28	0.10	1.45	14.17	7.99	0.037	0.088	0.144	0.022	0.021	0.031	0.050	0.017	3.7	0.021
		SD	0.02	-	-	-	0.18	0.003	0.013	0.020	0.003	0.003	0.004	0.003	0.002	-	-
	2	mean	4.50	0.10	1.52	15.39	8.41	1.306	0.123	0.234	0.039	0.015	0.067	0.100	0.021	3.3	0.044
		SD	0.03	-	-	-	-	0.065	0.001	0.002	0.000	0.001	0.002	0.004	0.001	-	-
	5	mean	5.46	0.12	1.69	13.79	7.91	0.053	0.078	0.147	0.037	0.019	0.038	0.059	0.018	6.0	0.088
		SD	0.01	-	-	-	0.13	0.002	0.010	0.019	0.005	0.002	0.011	0.009	0.001	-	-
Does not have ash influence	6	mean	5.07	0.13	1.94	15.33	7.88	0.074	0.079	0.100	0.032	0.020	0.064	0.070	0.027	0.5	0.066
		SD	0.02	-	-	-	0.12	0.009	0.009	0.011	0.004	0.002	0.041	0.028	0.001	-	-
	9	mean	5.13	0.09	1.23	14.19	8.16	0.364	0.103	0.180	0.028	0.020	0.052	0.070	0.018	1.6	0.022
		SD	0.03	-	-	-	0.15	0.040	0.011	0.021	0.006	0.002	0.003	0.001	0.001	-	-
	10	mean	5.66	0.13	1.77	13.94	8.10	0.019	0.113	0.171	0.026	0.027	0.046	0.056	0.017	6.6	0.033
		SD	0.01	-	-	-	0.31	0.001	0.005	0.014	0.002	0.003	0.008	0.006	0.001	-	-
	14	mean	4.85	0.11	1.72	15.50	8.20	0.155	0.109	0.196	0.026	0.025	0.057	0.071	0.020	1.6	0.027
		SD	0.06	-	-	-	0.04	0.006	0.007	0.012	0.002	0.002	0.028	0.018	0.001	-	-
	15	mean	6.12	0.28	3.37	11.92	8.10	0.001	0.148	0.312	0.060	0.033	0.040	0.105	0.022	5.5	0.034
		SD	0.03	-	-	-	0.43	0.000	0.006	0.014	0.003	0.001	0.000	0.001	0.001	-	-
	3	mean	5.87	0.13	1.96	15.61	10.10	0.041	0.540	0.359	0.062	0.165	0.172	0.073	0.021	19.5	0.042
		SD	0.06	-	-	-	0.09	0.001	0.056	0.035	0.005	0.020	0.004	0.004	0.003	-	-
	4	mean	5.97	0.11	1.84	16.80	9.32	0.013	0.351	0.356	0.059	0.096	0.121	0.090	0.022	14.1	0.026
		SD	0.05	-	-	-	0.10	0.000	0.018	0.018	0.002	0.005	0.004	0.004	0.002	-	-
	7	mean	6.14	0.15	3.10	20.17	9.04	0.003	0.227	0.350	0.109	0.042	0.083	0.102	0.050	17.8	0.065
		SD	0.03	-	-	-	0.06	0.000	0.050	0.076	0.025	0.009	0.004	0.002	0.000	-	-
Has ash influence	8	mean	6.25	0.21	4.94	23.64	9.35	0.007	0.338	0.321	0.108	0.082	0.168	0.163	0.063	18.4	0.022
		SD	0.07	-	-	-	0.24	0.000	0.015	0.014	0.005	0.002	0.003	0.003	0.001	-	-
	11	mean	5.58	0.11	1.34	12.69	8.58	0.134	0.155	0.312	0.039	0.028	0.087	0.101	0.020	10.5	0.093
		SD	0.04	-	-	-	0.17	0.006	0.021	0.054	0.008	0.005	0.035	0.019	0.003	-	-
	12	mean	6.05	0.12	1.59	13.17	8.04	0.005	0.126	0.419	0.071	0.028	0.035	0.074	0.024	6.9	0.033
		SD	0.02	-	-	-	0.14	0.000	0.011	0.040	0.004	0.004	0.011	0.013	0.004	-	-
	13	mean	6.30	0.11	1.54	13.45	10.18	0.030	0.611	0.385	0.062	0.209	0.144	0.044	0.008	18.9	0.012
		SD	0.02	-	-	-	0.11	0.001	0.035	0.034	0.004	0.013	0.001	0.003	0.001	-	-

Table 3.2. Subgroup and series-level classifications by USDA mapping of sampled site

				No significant ash influence observed				
Site	1	2	5	6	9	10	14	15
Mapped subgroup	Argiaquic Xeric Argialbolls	Vitrandic Fragixeralfs	Oxyaquic Argixerolls	Xeric Argialbolls	Vitrandic Argixerolls	Oxyaquic Argixerolls	Argiaquic Xeric Argialbolls	Pachic Ultic Argixerolls
Mapped series	Latahco silt loam	Carlinton Dry Taney Complex	Thatuna-Naff Complex	Tilma-Naff Complex	Taney ashy silt loam	Southwick silt loam	Latahco-Thatuna Complex	Jacket silt loam
Land use	Annual crop	Annual crop	CRP	Annual crop	Annual crop	Annual crop	Annual crop	Pasture

			Significant ash influence observed				
Site	3	4	7	8	11	12	13
Mapped subgroup	Vitrandic Fraglossudalfs	Vitrandic Fraglossudalfs	Vitrandic Fragixeralfs	Vitrandic Fragixeralfs	Vitrandic Fragixeralfs	Vitrandic Fragixeralfs	Vitrandic Fraglossudalfs
Mapped series	Reggear-Santa Complex	Reggear-Santa Complex	Santa ashy silt loam	Santa ashy silt loam	Santa ashy silt loam	Santa ashy silt loam	Reggear-Santa Complex
Land use	CRP	Planted forest	Logged forest	Native forest	Annual crop	Annual crop	CRP

Although sites 2 and 9 were mapped as Vitrandic, the samples taken from their surface did not meet requirement (3) and were placed in the "no significant ash influence" category. Figure 3.2 shows differences in oxalate-extractable metals, NaF pH, pH, Al_{KCl}, Al_p, and glass content between soils that met the experiment's threshold for ash influence and those that did not. Oxalate-extractable Al and Fe, Al_p, NaF pH, and glass, which are associated with ash mineralogy, were predictably higher in ash-influenced soils ($p < 0.05$). Soil pH was significantly higher and Al_{KCl} was insignificantly higher in ash-influenced soils.
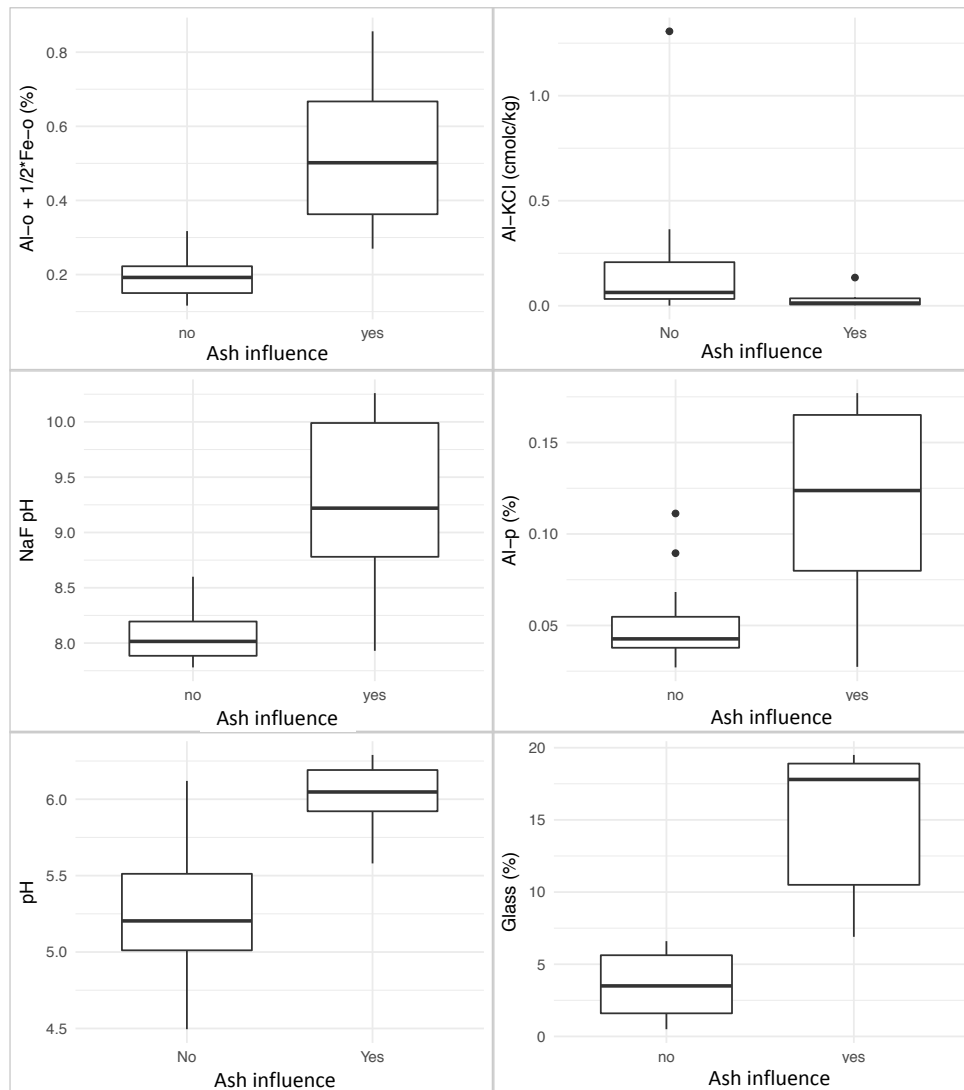
Figure 3.2. $Al_o + \frac{1}{2} Fe_o$, NaF pH, pH, Al_{KCl}, Al_p, and glass content in soils that met ash influence qualifications (n = 7) and those that did not (n = 8). Horizontal lines represent quartiles, vertical lines represent 1.58 times the interquartile range (which represents approximately a 95% confidence interval about the median. See McGill et al., 1978), and dots represent outliers.

Figure 3.3 shows the relationship between pH and Al_{KCl} in soils with and without ash properties. It is difficult to rigorously assess whether andic status changed the effect of pH on Al availability because we lacked ash-influenced soils at the low end of the pH range. Although the experiment was designed to sample both andic and non-andic soils across the same pH range, the soil thought to represent the lower end of the range for the andic category did not meet the qualifications for ash influence classification. When Al_{KCl} was modeled as a function of pH and andic status, the interaction was not significant (Table 3.3, $p > 0.05$).
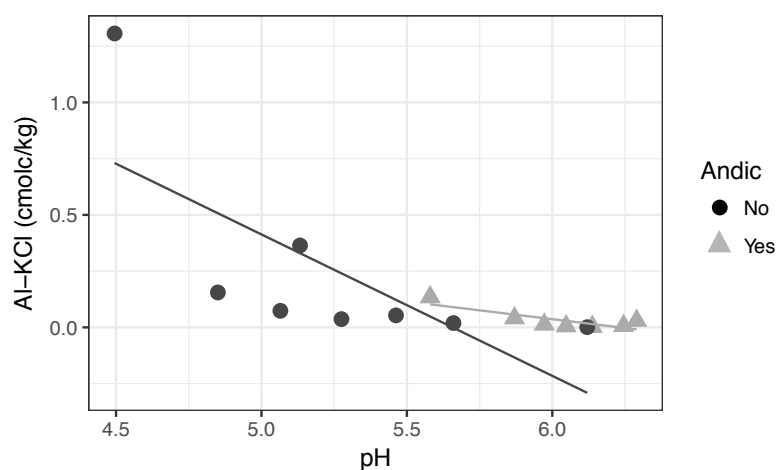
Figure 3.3. pH vs. KCl-extractable Al

Table 3.3. Al_{KCl} modeled as a function of pH, andic status, and their interaction (pH:Andic).

	Al_{KCl}	
Term	*Coefficient*	*p-value*
(Intercept)	3.56	0.004
pH	-0.63	0.007
Andic	-2.58	0.358
pH:Andic	0.47	0.322
R^2 / adj. R^2	0.563 / 0.443	

Since andic status is in reality based on a continuum of properties rather than a strict binary, available Al was also modeled on soil properties associated with volcanic ash. Predictors for the model were initially selected from the variables that showed the most correlation with Al_{KCl} but minimized correlation between one another. Table 3.4 shows a correlation matrix of soil properties.

Table 3.4. Spearman correlations between soil properties. *Significant at $p < 0.05$.

	pH	N	C	C:N	NaF pH	Al_{KCl}	Al_o	Fe_o	Mn_o	Si_o	Al_p	Fe_p	Mn_p	Glass
pH	1.00													
N	0.56*	1.00												
C	0.49	0.90*	1.00											
C:N	-0.03	0.10	0.44	1.00										
NaF pH	0.47	-0.07	0.08	0.46	1.00									
Al_{KCl}	-0.84*	-0.69*	-0.64*	0.03	-0.05*	1.00								
Al_o	0.75*	0.24	0.32	0.20	0.86*	-0.44	1.00							
Fe_o	0.72*	0.18	0.23	0.08	0.71*	-0.51	0.88*	1.00						
Mn_o	0.80*	0.53*	0.54*	0.21	0.55*	-0.66*	0.75*	0.82*	1.00					
Si_o	0.83*	0.49*	0.55*	0.20	0.65*	-0.64*	0.87*	0.74*	0.66*	1.00				
Al_p	0.40	0.13	0.29	0.55*	0.86	-0.01	0.76*	0.46	0.47	0.64*	1.00			
Fe_p	0.29	0.41	0.45	0.28	0.24	-0.34	0.41	0.41	0.58*	0.20	0.31	1.00		
Mn_p	0.23	0.58*	0.67*	0.44	0.02	-0.40	0.16	0.28	0.61*	0.17	0.12	0.72*	1.00	
Glass	0.81*	0.36	0.34	0.18	0.77*	-0.52*	0.88*	0.75*	0.72*	0.82*	0.67*	0.23	0.08	1.00

Soil pH, N, C, Fe_o, Mn_o, and volcanic glass content showed significant linear correlations with Al_{KCl}. C/N ratio, NaF pH, Al_o, Si_o, and glass correlated significantly with Al_p. However, some of these variables were unsuitable to use in multiple regression models because they were highly collinear. Collinearity is problematic because it can make the model very sensitive to changes, increasing the variance of the coefficient estimates. This increases the likelihood of rejecting the null hypothesis and causing a Type II error. Additionally, highly collinear variables may capture the same underlying process; adding a predictor to a model that already has a similar term reduces the model's statistical power without much improvement. It is also difficult to measure the individual effect of each collinear term.

Soil pH and Al_o were selected as predictors that gave the model the lowest mean square error (MSE) and collinearity (measured as variance inflation factor, or VIF) and maximized R^2 and goodness of fit, measured as corrected Aikake Information Criteria, or AIC_c (Hurvich, 2003). Surprisingly, Al_p did not correlate significantly with Al_{KCl}. Other predictors correlated well individually with Al_{KCl}, but were highly collinear or produced a worse model according to the metrics described above.

Regression assumes that the size and variance of residuals will stay the same for all values of the independent variable. Residuals that do not meet this assumption can bias the model and lead to faulty conclusions about the significance of regression coefficients. Transforming variables can bring the data into line with model assumptions. In this case, Al_{KCl} was log transformed. Table 3.5 summarizes the model.

Table 3.5. Al_{KCl} modeled as a function of soil pH and Al_o.

	log(Al_{KCl})		
Term	*Coefficient*	*p-value*	*VIF*
(Intercept)	7.466	<.001	
pH	-1.684	<.001	1.547
Al_o	2.278	0.008	1.547
MSE / AICc	1.639 / 21.356		
R^2 / adj. R^2	0.834 / 0.806		

Model predictors pH and Al_o were somewhat collinear, but still yielded VIF values below 5, which is considered satisfactory according to a common rule of thumb (Craney and Surles, 2002). Although soil C is thought to exert a negative effect on Al availability, it did not contribute much to the model's fit and was excluded (Vieira et al., 2008). While Al_o and Al_{KCl} were negatively correlated on their own, Al_o correlated positively with Al_{KCl} once pH was included in the model. This shows that one-to-one relationships can be confounded by other variables, and that accurately assessing the influence of soil properties on Al availability requires modeling their influence simultaneously. This model shows that at a given pH, Al_o exerts a positive influence on Al_{KCl}.

Although Al_{KCl} was treated as the main indicator of Al availability, some research has found the Al fraction bound to organic matter is plant available as well. This fraction is typically extracted with $CuCl_2$ or EDTA (Bertsch and Bloom, 1996; Mora et al., 2012). Although we did not perform these analyses, we investigated the influence of ash-associated properties on Al_p, which is thought to represent total organically bound Al. Table 3.4 shows Spearman correlations between soil properties and Al_p. Most variables were highly collinear. C/N ratio and either NaF pH or Al_o were adequate predictors in a multiple regression model of Al_p. Table 3.6 summarizes the models.

Table 3.6. Al_p modeled as a function of Al_o or NaF pH and C/N ratio.
*Significant at $p < 0.05$ ** Significant at $p < 0.01$.

	Al_p				Al_p		
Term	*Coef.*	*p*	*VIF*	*Term*	*Coef.*	*p*	*VIF*
(Intercept)	-0.423	<0.001		(Intercept)*	-0.053	0.039	
NaF pH**	0.052	<0.001	1.172	Al_o**	0.226	<0.001	1.067
C:N*	0.004	0.036	1.172	C:N**	0.006	0.003	1.067
MSE / AIC_c	0.003 / -72.8			MSE / AIC_c	0.003 / -75.6		
R^2 / adj. R^2	0.906 / 0.890			R^2 / adj. R^2	0.898 / 0.881		

Discussion

Soil pH and andic status did not appear to exhibit an interaction in their effects on Al_{KCl}—in other words, the effect of pH on Al_{KCl} did not depend on andic status in these data. More samples in the low end of the pH range might have changed this outcome. The soil samples thought to represent low-pH andic soils did not meet the threshold for significant ash

influence, meaning andic soils were not represented across the same pH range as non-andic soils.

This sampling procedure did not distinguish between allophanic and non-allophanic andic soils. Non-allophanic soils tend to contain more Al_{KCl}, more Al_p, and less Al_o (Johnson-Maynard et al., 1997). According to the classification used by Dai et al. (2011), 3 of the soil samples were non-allophanic (i.e. had a ratio of Al_p/Al_o greater than 0.5 or Al_p/Al_o less than 0.5 with more than 8 percent clay). However, these soils did not have particularly high Al_{KCl}, and Al_{KCl} did not correlate significantly with Al_p or Al_p/Al_o,. This aspect of andic mineralogy was therefore probably not associated with differences in Al bioavailability in this study.

Al_{KCl} correlated relatively well with pH, C, N, oxalate extractable Al, Fe, Mn, and Si, and glass content, and did not appear to be associated with C/N ratio, NaF pH, or pyrophosphate-extractable metals. Of these variables, pH, C, and Al_o best modeled Al_{KCl}, due in part to collinearity between the others. Al_{KCl} correlated negatively with pH. Once we controlled for pH by including it as a regression term, Al_o was positively correlated with Al_{KCl}. Evidence in the literature suggests that allophanic Andisols, which contain a large proportion of poorly crystalline Al, exhibit less Al toxicity than non-allophanic Andisols. For example, Ito et al. (2009) found that adding allophanic minerals to growth medium ameliorated Al toxicity to plants. Al_p was not correlated to Al_{KCl}, contrary to findings by Takahashi et al. (2011) of strong correlation between the two. The positive correlation between Al_o and Al_{KCl} and the absence of an Al_p-Al_{KCl} relationship suggest that the distinction between allophanic and non-allophanic mineralogy was not relevant to Al availability in this study. This is similar to observations by Yamada et al. (2011) of Al toxicity to plants in both allophanic and non-allophanic Andisols at low pH.

The positive correlation between Al_o and Al_{KCl} when controlling for pH contradicts the results of the interaction model of pH and andic status on Al_{KCl}, which showed that Al_{KCl} did not respond differently to pH change in andic and non-andic soils. Since andic soils contain more Al_o, they presumably show greater Al availability at lower pH. The contradiction may be explained by the somewhat arbitrary nature of the andic classification, which imposes a binary over continuous properties. It may also be due the fact that andic soils exhibited most of the variation in Al_o, while occurring over a small portion of the pH range (5.5-6.5). In inferring relationships from this limited pH range, the regression results may not

adequately explain the Al_o-Al_{KCl} relationship across the whole pH range accounted for in the interaction model. The continuous regression model's results may be more accurate because it relies on measured properties rather than a binary, even though it infers relationships from a more limited pH range. If this is the case, the binary classification may not be as useful a proxy for ash influence as initially conceived. The results of the continuous model, presented in Table 3.5, suggest that Al toxicity is more severe in low pH soils with ash influence.

Al_p correlated closely with NaF pH, Al_o, Si_o, C/N ratio, and glass content, but collinearity excluded most of these variables from the model. Al_o and NaF pH both predicted Al_p well, especially when C/N ratio was included as a predictor. The Al_o-NaF pH and Al_o-Al_p relationships are unsurprising, since organometal complexation and high NaF pH are associated with poorly crystalline mineral content. However, the weakly positive influence of C/N ratio on Al_p is counterintuitive, considering that lower C/N ratio is associated with more charged sites on soil organic matter, which may increase bonding with Al (Harada and Inoko, 1980). Other research investigating the effect of organic matter quality on Al availability has found that low C/N ratios were associated with more high-stability Al-humus complexes, while higher C/N was associated with more low-stability Al-humus complexes (Eimil-Fraga et al., 2015). Organic matter quality may be an important indicator of Al available through weak organometal complexes.

Conclusions

Aluminum toxicity due to soil acidification poses a growing threat to Northern Idaho agriculture. Understanding the influence of volcanic mineralogy on Al availability under acidifying conditions could help growers develop strategies to manage Al toxicity based on soil type. This study provided evidence that soils with volcanic ash influence, in the form of high levels of poorly crystalline Al oxides, influenced the relationship between soil pH and Al availability. Al_o, C, and pH produced the best model for Al_{KCl}, although other soil properties correlated well with Al_{KCl}. Al_o was positively correlated with Al_{KCl}, despite evidence in the literature that greater quantities of allophanic minerals are associated with less Al toxicity. This suggests that soils with ash influence could results in more severe Al toxicity at a given pH than soils without ash. However, since the ash-influenced soils were limited to pH range of 5.5-6.5, a broader range of pH values will be necessarily to evaluate the effect of ash mineralogy on Al availability. NaF pH, Al_o, and C/N ratio were the best predictors for

organically bound Al. Positive correlation between C/N ratio and Al_p suggests that organic matter quality plays a role in determining Al speciation.

The positive influence of Al_o on Al_{KCl}, when controlling for pH, suggests that growers may benefit from liming ash soils at higher pH than they would lime soils without ash. However, further study that addresses a wider pH range is necessary to assess whether ash mineralogy influences Al toxicity under acidification. Since there is some dispute about which Al forms are most toxic to plants, future research should investigate the effects of andic properties on other plant-available fractions, such as Al weakly bound to organic matter, or use plant growth trials to assess bioavailability.

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Appendix A

Table A.1. Soil physical properties used to calculate watering requirements. *Volumetric water contents at field capacity and permanent wilting point were assumed based on texture (Campbell 2008). Gravimetric water content is calculated using the equation $\theta_m = \theta_v \div \frac{\rho_b}{\rho_w}$, where θ_m is gravimetric water content, θ_v is volumetric water content, ρ_b is bulk density, and ρ_w is the density of water.

Dry soil weight (g)	485	
Bulk density (g/cm^3)	1.3	
	Field capacity	Wilting point
Volumetric water content*	0.27	0.13
Gravimetric water content	0.21	0.10

Table A.2. Recommended fertilizer rates (R. L. Mahler & Guy, 2007)

P calculation		N calculation	
Soil test P (NaOAc) (ppm)	Application rate (P2O5) (lbs/ac)	1. Total N needed for 160 bu/ac (lbs/ac)	300
0-2	60	2. Inorganic N (lbs/ac)	21
2-3	40	3. Mineralizable N (lbs/ac)	60
3-4	20	1 – (2 + 3) =	
Over 4	0	N applied (lbs/ac)	219

Table A.3. SRM digestion recovery rates

	Cd (mg/kg)	Cu (mg/kg)	Fe (%)	Mn (mg/kg)	P (%)	Zn (mg/kg)
Mean measured concentration	2.43	8.783	412	202	0.404	54.7
% of accepted SRM value	81%	80%	60%	85%	119%	89%

Table A.4. Complete results including in-house grain digests

P level	Cd (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	Cu (mg/kg)	Fe (%)	P (%)
P0-1	0.08	82.0	84	9.38	0.004	0.66
P0-2	0.03	60.4	77	8.98	0.007	0.53
P0-3	0.07	66.8	82	8.18	0.008	0.49
P0-4	0.06	55.1	73	6.46	0.004	0.42
P0-5	0.05	65.0	77	8.96	0.009	0.53
P0-6	0.08	73.1	76	8.9	0.008	0.51
P0-7	0.07	59.5	60	7.07	0.004	0.49
P1-8	0.05	61.9	83	9.28	0.011	0.48
P1-1	0.05	50.8	69	7.28	0.011	0.48
P1-2	0.06	60.6	76	7.44	0.010	0.48
P1-3	0.06	59.1	87	8.56	0.010	0.47
P1-4	0.09	56.3	82	8.08	0.011	0.408
P1-5	0.05	60.5	80	8.87	0.011	0.554
P1-6	0.05	54.8	77	8.09	0.01	0.546
P1-7	0.07	59.6	85	8.41	0.012	0.549
P1-8	0.05	59.7	73	7.45	0.011	0.497
P3-1	0.06	35.3	75	3.83	0.007	0.333
P3-2	0.07	52.7	91	6.22	0.013	0.508
P3-3	0.04	36.9	65	5.7	0.011	0.436
P3-4	0.08	39.9	61	4.79	0.008	0.399
P3-5	0.07	36.6	76	4.57	0.008	0.372
P3-6	0.08	42	57	4.8	0.008	0.405
P3-7	0.06	37.2	58	3.74	0.01	0.33
P3-8	0.07	36.6	71	3.86	0.005	0.386
P5-1	0.06	32.8	75	4.63	0.007	0.4
P5-2	0.07	40	76	4.14	0.007	0.433
P5-3	0.06	35.6	66	5.24	0.007	0.35
P5-4	0.05	38.4	67	4.83	0.007	0.39
P5-5	0.11	52.6	81	5.24	0.008	0.573
P5-6	0.08	42.9	76	3.81	0.007	0.394
P5-7	0.08	37.7	65	4.1	0.007	0.342
P5-8	0.1	45.5	71	4.32	0.008	0.461

Table A.5. Complete Mehlich extraction results. Some samples were analyzed in triplicate.

Soil fraction	P level	pH	Cd (mg/kg)	Fe (mg/kg)	Mn (mg/kg)	P (mg/kg)	Zn (mg/kg)
Unplanted	P0-1	5.39	0.05	257.05	107.1	28.585	1.87
Unplanted	P0-2	5.53	0.045	227.75	80.15	26.135	1.99
Unplanted	P0-3	5.53	0.055	235.25	101.95	30.28	2.385
Unplanted	P0-4	5.1	0.055	263.75	104.45	28.14	2.265
Unplanted	P0-5	5.66	0.045	235.2	75.2	27.64	1.8
Unplanted	P0-6	5.28	0.045	215.4	71.6	26.37	1.81
Unplanted	P0-7	5.26	0.05	251.25	85.6	32.015	2.1

Unplanted	P0-8	5.5	0.045	236.8	99.8	32.265	2.06
Unplanted	P1-1	5.38	0.05	246.1	101.7	30.775	2.06
Unplanted	P1-2	5.53	0.05	231.25	98.5	31.345	1.985
Unplanted	P1-3	5.45	0.05	258.25	101.55	35.41	1.95
Unplanted	P1-4	5.32	0.05	233.8	90.75	31.63	2.105
Unplanted	P1-5	5.34	0.05	239.8	90.4	35.105	2.065
Unplanted	P1-6	5.45	0.055	251.4	103.45	35.88	2.21
Unplanted	P1-7	5.2	0.055	248.75	109	30.71	2.235
Unplanted	P1-8	5.29	0.05	251.75	93.1	34.27	2.245
Unplanted	P1-8	5.29	0.05	241.05	94.7	32.87	2.215
Unplanted	P1-8	5.29	0.05	242.15	89.6	32.055	2.135
Unplanted	P3-1	5.27	0.055	244.05	97.45	46.425	2.555
Unplanted	P3-2	5.49	0.05	254.3	97.1	42.625	2.32
Unplanted	P3-3	5.19	0.06	264.85	92.8	47.985	2.485
Unplanted	P3-4	5.22	0.06	248.75	91.2	56.4	2.315
Unplanted	P3-4	5.22	0.06	246.5	92.8	57.7	2.3
Unplanted	P3-4	5.22	0.055	243.95	91.2	57.55	2.285
Unplanted	P3-5	5.57	0.06	254.8	93.85	60.95	2.475
Unplanted	P3-6	5.48	0.06	241.1	97.1	50.9	2.405
Unplanted	P3-7	5.49	0.055	243	94.1	52.35	2.45
Unplanted	P3-8	5.27	0.055	269.3	89.8	45.92	2.335
Unplanted	P5-1	5.52	0.055	248.25	84.25	63.5	2.41
Unplanted	P5-2	5.37	0.05	231.55	84.3	43.74	2.06
Unplanted	P5-3	5.19	0.055	248.7	91	43.72	2.145
Unplanted	P5-4	5.26	0.055	252.95	94.75	48.785	2.22
Unplanted	P5-5	5.46	0.065	256.15	106.55	66.5	2.755
Unplanted	P5-6	5.5	0.055	247.55	95	49.44	2.29
Unplanted	P5-7	5.52	0.055	265.8	100.95	56.45	2.325
Unplanted	P5-8	5.55	0.065	269.05	107.35	50.5	2.715
Rhizosphere	P0-1	5.56	0.06	280	143.75	25.8	2.33
Rhizosphere	P0-2	5.94	0.055	264.75	117.8	24.655	2.205
Rhizosphere	P0-3	5.31	0.055	324.2	111.55	24.895	2.19
Rhizosphere	P0-4	5.11	0.05	239.95	109.1	27.025	1.9
Rhizosphere	P0-5	5.82	0.055	260.75	97.6	27.67	2.295
Rhizosphere	P0-6	5.23	0.06	270.1	99.2	25.975	2.055
Rhizosphere	P0-6	5.23	0.06	256.65	96.3	24.14	2.01
Rhizosphere	P0-6	5.23	0.065	246.5	133.5	25.325	1.93
Rhizosphere	P0-7	5.09	0.055	248.55	117.4	29.395	1.95
Rhizosphere	P0-8	5.5	0.045	201.7	107.35	25.71	1.535
Rhizosphere	P1-1	5.79	0.05	245.25	115.6	26.1	1.875
Rhizosphere	P1-2	5.76	0.055	277.85	108	28.05	2.1

Rhizosphere	P1-3	5.58	0.055	262.7	114.25	27.82	1.955
Rhizosphere	P1-4	5.37	0.05	276.6	81.15	23.53	2.24
Rhizosphere	P1-5	5.76	0.04	207.6	90.5	26.135	1.33
Rhizosphere	P1-6	5.85	0.045	240.65	96.8	27.225	1.7
Rhizosphere	P1-7	5.52	0.05	224.3	115.95	24.36	1.685
Rhizosphere	P1-8	5.9	0.045	245.2	83.55	27.765	1.7
Rhizosphere	P3-1	5.77	0.055	260.35	106	38.805	2.155
Rhizosphere	P3-2	5.79	0.055	280.15	89.5	33.18	2.1
Rhizosphere	P3-3	5.73	0.055	266.45	107.95	37.4	2.31
Rhizosphere	P3-4	5.54	0.05	263.6	77.95	40.57	1.875
Rhizosphere	P3-5	5.67	0.05	238.55	78.1	36.075	1.83
Rhizosphere	P3-5	5.67	0.045	255.05	85.95	35.52	1.82
Rhizosphere	P3-5	5.67	0.05	250.25	81.8	36.125	1.815
Rhizosphere	P3-6	5.69	0.065	279.9	114.55	39.95	2.57
Rhizosphere	P3-7	5.64	0.055	254.4	92.35	42.18	1.98
Rhizosphere	P3-8	5.48	0.055	273.95	101.05	37.33	2.275
Rhizosphere	P5-1	6.08	0.05	225.65	81.35	36.34	1.785
Rhizosphere	P5-2	5.83	0.055	298.7	92.85	36.835	1.985
Rhizosphere	P5-3	5.99	0.05	250.35	73.8	36.58	1.785
Rhizosphere	P5-3	5.99	0.045	261.4	73.1	36.85	1.73
Rhizosphere	P5-3	5.99	0.045	243.6	71.25	34.91	1.69
Rhizosphere	P5-4	6.07	0.055	261.6	108.75	43.21	2.36
Rhizosphere	P5-5	5.96	0.055	243.3	103.1	39.475	2.21
Rhizosphere	P5-6	5.74	0.06	240.9	98.05	43.24	2.055
Rhizosphere	P5-7	5.59	0.055	245.25	74.75	41.115	1.775
Rhizosphere	P5-8	5.62	0.055	294.35	82.85	44.365	1.795
Bulk	P0-1	6.38	0.06	249.278	135.575	42.521	2.227
Bulk	P0-2	5.61	0.05	232.684	110.095	34.013	1.714
Bulk	P0-3	5.86	0.07	265.07	153.393	34.247	2.54
Bulk	P0-4	5.31	0.07	278.774	133.533	59.342	2.601
Bulk	P0-5	5.51	0.059	248.916	134.581	38.793	2.236
Bulk	P0-6	5.66	0.065	264.556	154.337	38.315	2.243
Bulk	P0-7	5.29	0.06	245.681	146.48	38.497	2.112
Bulk	P0-8	5.57	0.065	265.442	148.064	37.438	2.329
Bulk	P1-1	5.97	0.064	269.955	122.707	57.164	2.37
Bulk	P1-2	5.78	0.07	269.189	139.423	59.333	2.399
Bulk	P1-3	5.79	0.055	244.339	122.444	34.085	2.07
Bulk	P1-4	5.37	0.07	263.691	150.873	46.185	2.564
Bulk	P1-5	5.62	0.07	260.08	159.88	42.465	2.211
Bulk	P1-6	6.1	0.06	264.129	132.587	38.338	2.239
Bulk	P1-7	5.6	0.065	250.175	159.511	35.671	2.406

Bulk	P1-8	5.57	0.065	258.172	159.215	36.448	2.375
Bulk	P3-1	6.11	0.06	235.982	96.702	48.306	2.349
Bulk	P3-2	5.87	0.06	250.822	141.654	36.577	2.267
Bulk	P3-3	6.45	0.056	224.785	92.327	42.12	2.246
Bulk	P3-4	6.13	0.069	274.691	129.214	58.675	2.392
Bulk	P3-5	5.99	0.059	232.314	98.323	48.337	2.304
Bulk	P3-6	5.74	0.069	274.665	154.933	53.049	2.776
Bulk	P3-7	6.24	0.055	212.412	94.745	39.349	2.232
Bulk	P3-8	5.93	0.069	249.975	152.842	43.114	2.333
Bulk	P5-1	6.32	0.07	264.094	127.888	63.596	2.555
Bulk	P5-2	6.02	0.054	227.613	121.893	33.323	2.096
Bulk	P5-3	5.93	0.07	276.858	131.82	59.401	2.564
Bulk	P5-4	6.18	0.075	269.526	149.776	51.82	2.703
Bulk	P5-5	6.11	0.069	278.405	125.062	55.225	2.432
Bulk	P5-6	5.84	0.075	288.667	159.511	55.866	2.636
Bulk	P5-7	5.66	0.08	285.971	133.849	64.403	2.676
Bulk	P5-8	5.94	0.065	266.4	148.7	34.53	2.355

Table A.6. Mass of grain, stems, leaves, and roots.

Sample	Mass (g)		
	Grain	Stems/leaves	Roots
P0-1	0.444	0.631	0.26
P0-2	0.688	0.68	0.773
P0-3	0.904	0.772	0.683
P0-4	0.48	0.535	0.753
P0-5	0.593	0.504	0.452
P0-6	0.715	0.662	0.633
P0-7	0.474	0.485	0.48
P0-8	0.559	0.644	0.471
P1-1	0.907	0.797	0.439
P1-2	0.832	0.802	0.498
P1-3	0.962	0.903	0.458
P1-4	0.839	0.743	0.292
P1-5	0.772	0.851	0.353
P1-6	0.859	0.845	0.391
P1-7	0.928	0.787	0.514
P1-8	0.841	0.733	0.434
P3-1	1.548	1.232	0.726
P3-2	1.04	1.405	0.589
P3-3	1.185	1.09	0.516
P3-4	1.289	1.351	0.515
P3-5	1.364	1.112	0.523
P3-6	1.254	1.255	0.602
P3-7	1.46	1.233	0.437
P3-8	1.394	1.343	0.441
P5-1	1.611	1.344	0.481
P5-2	1.467	1.538	0.495
P5-3	1.356	1.275	0.554
P5-4	1.332	1.367	0.412
P5-5	1.037	1.376	0.458
P5-6	1.654	1.714	0.326
P5-7	1.658	1.477	0.722
P5-8	1.705	1.568	0.389

Appendix B

Table B.1. Summary of grain element concentrations by variety

	Fe (mg/kg)		Mn (mg/kg)		Cu (mg/kg)		Zn (mg/kg)		Cd (ug/kg)		P (%)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Brundage 96	52.2	13.91	48.0	15.04	4.08	1.69	24.5	8.17	51.5	32.7	0.281	0.0330
LCS Artdeco	45.7	12.80	44.3	18.08	3.65	1.78	22.7	9.27	45.0	44.3	0.248	0.0229
LCS Star	45.7	8.94	38.8	5.96	4.40	0.94	27.4	7.52	144	71.9	0.304	0.0270
Norwest 553	44.0	13.64	45.8	12.21	2.92	1.78	29.3	10.56	45.7	40.5	0.273	0.0161
UI Pettit	34.8	3.77	38.2	5.38	2.84	2.06	32.1	6.57	119	83.7	0.334	0.0353
UI Platinum	52.0	8.37	45.2	7.07	4.08	0.63	30.0	5.51	111	45.7	0.338	0.0267
UI Silver	53.3	6.17	40.8	10.05	3.91	1.02	23.5	8.09	41.0	34.1	0.258	0.0275
UI Stone	52.4	24.28	53.6	20.40	3.15	1.60	31.4	7.79	105	68.9	0.302	0.0309
UI-WSU Huffman	46.9	11.38	47.4	21.55	4.32	0.95	21.0	7.46	36.9	29.8	0.258	0.0316
WB Junction	49.2	11.23	49.3	15.76	3.66	1.55	22.3	7.51	70.4	60.0	0.266	0.0361

Table B.2. PCs 7-12 of the soil dataset. "_d" refers to DTPA-extractable and "_t" to total soil concentrations.

	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
pH	-0.022	0.130	-0.068	0.534	0.568	-0.219	0.269	0.030
MAAT	0.539	0.330	-0.207	0.009	-0.108	-0.301	0.136	0.017
MAP	-0.536	-0.177	-0.022	0.143	-0.014	-0.507	0.117	0.018
Cd_d	0.027	0.474	0.064	0.100	0.094	-0.253	-0.479	0.201
Cu_d	-0.405	0.337	0.281	-0.294	-0.098	-0.094	0.067	-0.090
Fe_d	-0.249	0.316	-0.518	0.087	-0.058	0.014	0.267	-0.045
Mn_d	0.223	-0.229	0.553	-0.026	0.135	-0.400	0.191	-0.080
Zn_d	0.106	-0.526	-0.315	0.197	0.103	0.052	-0.142	0.039
Cd_t	0.086	0.020	0.018	-0.118	-0.118	0.104	0.669	-0.198
Zn_t	0.080	0.154	0.044	0.374	-0.206	0.111	-0.127	-0.667
Cu_t	0.020	0.108	0.177	0.243	-0.187	0.291	0.257	0.661
Mn_t	0.091	0.047	-0.258	-0.570	0.522	-0.026	0.038	-0.016
P_t	0.314	0.117	0.124	0.025	0.018	-0.070	0.058	0.016
Fe_t	-0.115	0.157	0.284	0.110	0.500	0.506	-0.001	-0.134
Standard deviation	0.624	0.407	0.393	0.310	0.259	0.154	0.119	0.061
Proportion of variance	0.028	0.012	0.011	0.007	0.005	0.002	0.001	0.000
Cumulative proportion	0.963	0.974	0.985	0.992	0.997	0.999	1.000	1.000

Table B.3. PCs 7-13 of the combined soil-grain dataset. "_g" refers to grain concentrations, "_d" to DTPA-extractable soil concentrations, and "_t" to total soil concentrations.

	PC7	PC8	PC9	PC10	PC11	PC12	PC13
Cd_g	0.015	0.295	-0.279	-0.243	0.109	0.309	-0.145
Cu_g	-0.326	-0.440	0.215	0.122	0.238	0.073	0.000
Fe_g	-0.278	-0.218	-0.479	-0.210	-0.042	0.000	-0.056
Mn_g	0.118	0.138	0.041	-0.123	0.061	-0.191	-0.126
P_g	-0.128	-0.179	-0.129	-0.012	0.290	0.233	0.575
Zn_g	-0.275	-0.219	0.252	0.074	-0.105	0.417	-0.191
Cd_d	0.192	-0.232	0.132	-0.280	0.098	0.162	0.258
Cu_d	-0.145	0.257	-0.210	-0.246	0.238	0.127	-0.179
Fe_d	-0.349	0.049	-0.267	0.351	-0.156	0.044	0.037
Mn_d	-0.112	0.030	0.429	-0.313	-0.076	-0.022	0.233
Zn_d	-0.092	-0.250	-0.335	0.121	0.024	-0.284	0.008
Cd_t	0.285	-0.237	0.120	0.069	0.078	-0.257	-0.229
Cu_t	-0.140	0.021	0.123	-0.094	0.058	-0.067	-0.168
Fe_t	0.002	0.029	0.031	-0.056	-0.073	0.504	-0.161
Mn_t	0.478	-0.374	-0.237	0.250	-0.028	0.224	-0.052
P_t	-0.301	-0.280	-0.028	-0.380	-0.400	-0.266	-0.103
Zn_t	-0.103	0.311	0.033	0.328	-0.345	-0.048	0.438
MAP	-0.119	0.068	-0.029	-0.020	0.615	-0.246	0.132
MAAT	0.253	-0.096	-0.217	-0.395	-0.247	0.007	0.330
Standard deviation	0.917	0.780	0.536	0.348	0.331	0.219	4.72E-16
Proportion of variance	0.044	0.032	0.015	0.006	0.006	0.003	0
Cumulative proportion	0.938	0.970	0.985	0.992	0.997	1.000	1

Table B.4. Locations of selected varieties.

Location	Brundage 96	UI Platinum	UI Pettit	UI Silver	UI Stone	LCS Star	LCS Artdeco	Norwest 553	UI-WSU Huffman	WB Junction
Bonners Ferry	x			x			x	x	x	x
Genesee	x			x			x	x	x	x
Moscow	x			x			x	x	x	x
Nezperce	x			x			x	x	x	x
Parker	x				x					
Southwick									x	
Tammany	x			x			x	x	x	x
Tensed	x			x			x	x	x	x
Aberdeen	x	x	x	x	x	x	x	x		x
Ashton		x	x		x	x				
Idaho Falls		x	x		x	x				
Kimberly	x		x		x		x	x		x
Parma					x					
Rexburg					x					
Rire	x			x				x		
Rockland				x				x		
Rupert		x				x		x		
Soda		x	x	x	x	x				
Tetonia			x		x					

Appendix C

Table C.1. Recoveries of oxalate and pyrophosphate extractions on known reference soils. Samples with recovery rates below 85% were Al_o in soil 2, Fe_o in soils 1 and 2, Fe_p in soil 2, Mn_o in soil 2, Mn_p in soils 1 and 2, and Si_o in soils 1 and 2.

		Reference soil 1			
Oxalate		Al (%)	Fe (%)	Mn (%)	Si (%)
	Measured	0.476	0.500	0.077	0.103
	Reference mean	0.550	0.667	0.087	0.149
	Recovery (%)	86%	75%	89%	69%
Pyrophosphate	Measured	0.158	0.071	0.014	
	Reference mean	0.170	0.080	0.017	
	Recovery (%)	93%	89%	83%	
		Reference soil 2			
Oxalate		Al (%)	Fe (%)	Mn (%)	Si (%)
	Measured	0.226	0.458	0.074	0.032
	Reference mean	0.303	0.641	0.091	0.057
	Recovery (%)	75%	71%	82%	56%
Pyrophosphate	Measured	0.071	0.075	0.017	
	Reference mean	0.082	0.118	0.024	
	Recovery (%)	87%	64%	72%	