Effects of Soil Properties and Wheat Variety on Cadmium Concentrations in Wheat Grain Grown in Idaho

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Abstract

Cadmium accumulation in wheat (*Triticum aestivum* L.) is a major pathway for human exposure causing risks to human health. The availability of Cd for uptake for wheat grains varies significantly with plant and soil properties. Idaho is one of the major wheat-growing areas in the United States, and it is important to investigate the regions and varieties that produce ultra-low Cd wheat grain to minimize health risk and improve the quality of food products. To discover the relationship between Cd uptake by wheat and soil properties and variety, grain and soil samples were collected from both southern and northern Idaho fields and metal content of grain and soils were measured, as well as soil properties. The main soil properties that affect Cd uptake by wheat were determined using three different regression models: Stepwise regression, Random Forest, and Partial Least Square. Means from different wheat varieties grown in different regions were evaluated to determine differences.

Almost all the wheat varieties grown in Idaho are safe for adult consumption, but most of them exceed the maximum allowable limit of Cd in infant food which is 0.04 mg/kg (EFSA, 2014). Among 65 different wheat varieties, UI Petit, a soft white spring wheat cultivar, had the highest Cd concentration, while UI Sparrow, a soft white winter wheat, had the lowest Cd concentration. The regression models revealed that DTPA Cd, Olsen P, total Cd, DTPA Zn, total Zn, total P, total N, NO_3^-—N, and organic carbon were significant soil measurements related to wheat grain Cd concentrations. Among them, DTPA Cd and Olsen P were identified as the most significant predictors for wheat grain Cd concentration in Idaho. Results from this research suggest that Cd bioavailability is one of the best predictors for Cd concentration in wheat grain, and management of phosphorous fertilization application is required to achieve low-Cd concentrations. The modeling approach was shown to be accurate when applied to other varieties and locations in Idaho.

Acknowledgements

My first and foremost gratitude is dedicated to my advisor Dr. Daniel Strawn for all his kind guidance, constant encouragement and valuable support throughout the research study.

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Dedication

This thesis is dedicated to my parents and family who have been always there to lift me up and encourage my every step and without their support I could not have completed this project.

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Chapter 1: Data analysis of soil-grain Cd concentrations to determine regions and varieties that are optimum for producing ultra-low Cd concentrations in wheat in Idaho.

Introduction

Wheat (*Triticum aestivum* L.) is a staple food in both developed and developing countries, on par with corn and rice in terms of the caloric importance (World Atlas, 2021). It provides Americans with more than half their daily intake of iron, thiamin, and folate, nutrients essential for energy and good health. With a predicted world population of 9 billion in 2050, the demand for wheat is expected to increase by 60% (GCARD, 2012). Production of consumable, safe, high-quality wheat is important to meet this demand. However, like most food crops, wheat uptakes cadmium (Cd), and because of its importance as a food source, it is a significant source of Cd in the human diet.

Cadmium is naturally present in soil and is also added to the soil as a contaminant leading to increased risks of uptake into plants used for food. Uptake of Cd by plants is highly variable, depending on plant characteristics and environmental conditions, and also depends on crop management practices. Thus, to reduce Cd exposure, information on factors affecting Cd uptake into plants is needed.

Cadmium Concentrations in Soils

Cadmium in soils is derived from natural and anthropogenic sources. Natural sources of Cd are associated with soil particles, bush and forest fires, sea salts, and volcanoes (ATSDR, 2012). Worldwide, the average abundance of Cd in soil is 0.36 mg/kg, and it varies between continents, countries, and soil type (Kubier et al., 2019). For instance, average concentration of Cd in USA is 0.27 mg/kg and in Australia it is 0.01 mg/kg (Holmgren et al., 1993).

A major source of Cd in soils occurs from anthropogenic activities. Phosphate fertilizer and fossil fuel combustion have been identified as major sources of Cd contamination, contributing 41% and 22% respectively of the Cd intake by humans from industrial processes (Van Assche, 1998). Other industrial and human activities like mining, smelting, electroplating, automobile exhaust, and battery production are associated with Cd contamination, which has a potential for dispersion in the environment through the air (Niragu, 1996; CSEM, 2008). For example, mining and smelting areas in England and Wales have caused 4,000 km^2 or more of soils to be contaminated by metals, including Cd (Thornton, 1997). Tobacco smoking is also a source of Cd for human exposure.

Environmental pollution occurs from contamination of the environment with waste generated by human activities (Ahmadpour et al, 2012). Since Cd is a non-degradable heavy metal, soil accumulates Cd in the environment (Moulis and Thevenod, 2010). Soil is polluted with Cd by fertilizers, sewage, and sludge. Composted sludge contains a high level of Cd (Kirkham, 2006). In the majority of contamination circumstances, wastes were applied directly to lands without any form of treatment. This has an impact on the environment along with implications for public health. Additionally, Cd can easily contaminate the natural environment due to its widespread distribution and high mobility (Moreno-Caselles et al., 2000).

Cadmium Toxicity and Exposure

Cadmium is a toxic heavy metal that serves no physiological function for any biological organism. Cadmium is absorbed into the human body through inhalation, digestion, and dermal exposure. Significant amounts of Cd accumulate in the liver and kidney (CSEM, 2008). Cadmium is a cumulative toxin, and it is transported in the blood, bound to proteins such as metallothionein and albumin. When Cd reaches the liver, it induces metallothionein production, eventually causing tubule cell necrosis (Godt et al, 2006). These mutations may lead to acute and chronic disorders. Permanent decreases in IQ can result from consuming Cd. A neurodevelopment study observed that increased risk of future criminal and antisocial behavior in children due to exposure to toxic heavy metals, including Cd (Rodríguez-Barranco et al., 2013). A study conducted in Sweden found that relatively low Cd exposure through the diet and smoking increases the risk of low bone mineral density and osteoporosis related fractures in elderly men (Wallin et al., 2016).

Cadmium accumulation from food is a major pathway for human exposure. Schwarz (1993) found that a German citizen has a daily intake of 30–35 μg of Cd and 95% of this is taken up with food and drinks. Bread and cereals contain high amount of Cd followed by potatoes, vegetables. Animals (mammals, fish, crustaceans, and mollusks) also contain Cd (Moulis and Thevenod, 2010). People who consume crops like wheat and maize grown in Cd-polluted soils are at risk of Cd exposure due to the bioaccumulation and translocation of Cd through the food chain (Yang et al, 2014).

The European Food Safety Authority (EFSA) has reviewed maximum contaminant levels for Cd in food (Jarup & Akesson, 2009). Based on a meta-analysis of 35 toxicological studies of European citizens, average daily allowable Cd intake was set at 0.00036 mg/kg body weight (~1/3 the WHO standard (WHO, 2003)). The study found that most of the population was close to or slightly exceeding the tolerable intake level, and some at risk groups (vegetarians, children, and smokers) exceeded the levels. As such, EFSA recommends decreasing Cd allowed in foods; first targeting wheat and rice because they are major dietary sources of Cd (grains account for 26.9% of total Cd intake from food) ((Jarup & Akesson, 2009; European Food Safety Authority, 2012).

Plant Uptake of Cd

Wheat genotype, soil properties and application of fertilizers affect Cd levels in wheat. The accumulation of Cd in grains varies due to the genetic differences, and may be due to variances in a plant's ability to transfer Cd from root to shoot (i.e., translocation) (Harris and Taylor, 2013; Kubo et al, 2016). For example, uptake of Cd is affected by plant root distribution (Liang et al, 2017), as well as Cd transporters regulating processes of trans-membrane transport (Ishimaru et al, 2012). Among the transporters, Nramp5 transporter protein is a major mechanism for Mn and Cd uptake in root cells (Sasaki et al, 2012).

A study conducted in the Netherlands on Cd contents in wheat found that Cd concentration ranged from 0.02 mg/kg to 0.35 mg/kg and some samples with high Cd levels come from contaminated river flood plains (Wiersma et al., 1986). The average amount of Cd in wheat grain grown in irrigated lands in Iran was reported as 0.91 mg/kg due to the high availability of Cd (Rahimi et al., 2021). In another study, among 16 different wheat cultivars grown in China, four had high concentrations of Cd that exceeded the maximum level of 0.2 mg/kg (EC, 2014); the highest Cd concentrations were 0.25 mg/kg (Guo et al., 2018). Most plants uptake Cd from soil. Even soils that have low levels of soil Cd can allow plant uptake. A study conducted in the Netherlands found that the average Cd uptake by wheat was 0.092 mg/kg in calcareous soils (Brus et al., 2005). Wenzel et al. (1994) reported high Cd accumulation in spring Durum wheat grown in agricultural soils in Austria (range was 0.18- 0.42 mg/kg). Kabata-Pendias (2000) summarized the reported Cd concentrations in wheat in different countries. For instances, in Australia, Egypt, Germany, U.S., and Russia, it was ranged as 0.0012-0.36, 0.01-0.09, 0.03-0.04, 0.07-0.13, and 0.06-0.07 mg/kg, respectively.

Research has shown that different varieties of the same plant species can have varying uptake affinities for Cd. These variances depend on plant biochemistry and physiology (Rizwan et al, 2016), and properties of the soil that the plant is grown in. As a result of genetic work coupled with breeding programs, Cd uptake in durum wheat (*Triticum turgidum* L. var *durum*) grown in Canada has been reduced by about 50% over a period of 40 years (Clarke et al, 2010; Penner et al, 1995). Uptake of Cd in durum wheat is controlled by the *Cdu1* gene, which has been localized to chromosome 5BL (Grant et al, 2008; Wiebe et al, 2010). Durum is a tetraploid species, while common wheat is a hexaploid species. Thus, genetic markers developed for durum wheat cannot be used to discover genes controlling Cd uptake in common wheat. However, given the variety uptake variance observed in common wheat, and the discovery of specific genes that can be exploited in breeding programs for durum wheat, it is likely that such genes exist in common wheat.

Soil factors, such as pH, organic matter content, cation exchange capacity (CEC), chloride, and phosphorus, affect Cd uptake by wheat. Among them, soil pH is an important factor that affects Cd availability for plant uptake (Page et al., 1987). At low pH, Cd mainly exists as Cd^{2+} ions, and adsorbed Cd is displaced from sites on soil particles by aluminum ions and protonA, making exchangeable Cd available for uptake by wheat (Nylund, 2003). Smolders (2001) observed that Cd uptake is mainly affected by chloride salinity, zinc-deficiency, and soil acidity.

Cadmium Regulations in Food

International and national food standards are established to ensure food safety for human health and development. The limitations and permissible levels of potential contaminants in food commodities are reported under those standards. Current EPA regulations for Cd intake from food are 0.001 mg/kg body weight per day (USEPA, 1999) (based on an ASTDR evaluation (ATSDR, 2008), which is the same value recommended by the FAO/WHO Expert Committee on Food Additives). Maximum Cd concentrations in food products are not currently regulated in the US, but international recommendations are supported. The FAO Codex Alimentarius recommended maximum allowable Cd in wheat is 0.2 mg/kg (dry weight). Due to the globalization of food distribution, most international food companies abide by this recommendation (Alexander et al, 2009). According to the EFSA (2014), for processed cereal-based food and baby food for infants and young children, maximum Cd levels allowable in wheat are set to 0.04 mg/kg. This marks a trend in awareness of Cd food safety risk and decreasing food safety standards, particularly for children and infants (Eklund and Oskarsson, 1999, Jean et al., 2018; Sanders et al., 2015; Taylor et al., 2014). A recent study conducted by the U.S. House of representatives (2021) reported that commercial baby foods are tainted with the high level of toxic heavy metals such as As, Cd, Hg, and Pb. As an example, Beech-Nut Company used 105 ingredients in its baby food that tested over 20 µg/kg Cd, and some tested much higher, up to 344.55 µg/kg Cd. As a response to this study, Teresa Murray, U.S. PRIG Education Fund Consumer Watchdog stated that baby food manufacturing companies should adhere to the highest of high standards and the federal government should adopt stricter standards to protect babies (U.S. PRIG, 2021). The Arsenic content in the product of Beech-Nut grain rice cereal was reported as above the limit of guidance level set by the FDA in August 2020 and the company decided to exit the market for Beech-Nut branded Single Grain Rice Cereal (US Food and Drug Administration, 2021).

Idaho wheat market

Wheat Production Regions

In terms of total acres, wheat is the number one crop in Pacific Northwest (PNW) States. The PNW is a productive wheat growing region, and breeding and variety development have allowed Idaho, Oregon, and Washington farmers to produce quality wheat (Ellis, 2019). In 2020, Washington, Idaho, and Oregon produced 72.5 Bu/Acre, 96.7 Bu/Acre, and 64 Bu/Acre of wheat, respectively (USDA-NASS, 2020).

Idaho is one of the major wheat-growing areas in the United States; ranked as 5^{th} leading wheat production state, with about 217.7 million bushels produced in 2020 (Statista, 2020). Idaho ranked 5^{th} in spring wheat production and 8^{th} in winter wheat production in the United States (USDA, 2012). According to the USDA, NASS, 2019 records, production of winter wheat is at 57.3 million bushels across 690,000 acres of harvested area. In 2020, winter, spring and durum wheat were harvested in 660,000, 495,000, and 9000 of acres respectively in Idaho (USDA-NASS, 2020).

Wheat Varieties

In Idaho, the farmers grow five different classes of wheat for domestic and international markets. Each class of wheat has unique characteristics for milling and end use, and also has varying agronomic practices. The five classes are hard red winter wheat (HRW), hard red spring wheat (HRS), hard white wheat, soft white wheat, and durum wheat. Hard white wheat and soft white wheat can be planted in either spring or winter varieties; in this chapter we use the notation SWW and HWW to indicate winter wheat varieties and SWS and HWS to indicate spring wheat varieties. Hard red winter wheat is mainly used for flour and cereal, flour tortillas, hard rolls, and flatbreads. Artisan and "designer" wheat foods like hearth bread, baguettes, croissants, bagels, and pizza crust are made by using hard red spring wheat and this is also blended with other flours to improve baking characteristics. The applications of hard white wheat are the production of whole wheat bread, Asian-style noodles, bagels, and hard-rolls. As low moisture wheat, soft white wheat can be identified with high extraction rates, providing a whiter, brighter product desired for cakes, pastries, cookies, crackers, and Asian-style noodles. Durum is the hardest kernel of the wheat classes, with the largest kernel and high protein content. It is used to make pasta, couscous, and Mediterranean bread (Lewin et al, 2013).

Marketing

Wheat is mainly grown in four regions in Idaho: North, Southcentral, Southwest, and Southeast. In Idaho, wheat is the dominant grain in all regions, ranging from 75% in the Southwest to 90% in the Southcentral (Jessup and Casavant, 2007). Soft white wheat is the most common wheat class, which is grown mostly in North and Southeast regions, comprising about 50% of total wheat production, while hard red spring wheat is mostly grown in the Southeast region (20% of the total grain production). Hard red winter wheat is grown in all regions of the state except the Southwest (Jessup and Casavant, 2007).

An estimated 10% of Idaho's wheat crop leaves the state as a value-added product (Lewin et al, 2013). Idaho wheat is shipped as a bulk commodity to 26 states in United States. Soft white wheat is exported to Asia. Buyers from Russia, China, Japan, Morocco, and Poland purchase hard red winter wheat. Taiwan millers typically purchase soft white wheat from Idaho, but are buying more hard red wheat in response to increased demand for bread. Most of the wheat in northern Idaho is exported to the Pacific Rim countries, with the Philippines, Taiwan, South Korea and Japan being the largest purchasers of soft white wheat (Lewin et al, 2013). An objective of this chapter is to identify regions in Idaho amenable for producing wheat grain that has the lowest Cd content. This information is needed to meet market demand for low-Cd food products.

Research goals

Cadmium accumulation in food is becoming a worldwide concern, and there is a need to supply low-Cd wheat to improve food safety. Wheat is an important and historic crop in Idaho. To meet market demand for low Cd wheat, University of Idaho, Idaho Wheat Commission, and wheat industry collaborators have embarked on a statewide survey of Cd concentrations in wheat grain and soils in Idaho's wheat growing regions. Paired soil and wheat grain samples were collected from 2014 to 2020. The target Cd grain concentration for use in baby food is 0.04 mg/kg, however the market has set a lower threshold of 0.03 mg/kg to provide a margin of safety. This low Cd-containing wheat is termed ultra-low Cd wheat grain.

This study was carried out to determine Cd concentrations in both soil and wheat grain in Idaho using data collected from 2014 to 2020. Data analysis was conducted to identify the relationships between soil and grain Cd, considering wheat varieties and other metal concentrations in both soil and wheat grain. In order to cover as many locations as the sampling capacity allowed in the four years, sampling sites and strategies varied each year. While this sampling limits data interpretation because it is not a repeated sample soil-grain analysis, the data can be used to get an overall survey of Cd distribution in both soil and wheat grain in different locations in Idaho.

Methods

Sampling

Location

Soil samples were collected at various locations across State of Idaho from 2014 to 2020. At each location, four to seven points were chosen for soil samples taken at depths of 0-15, 15-30, and 30-45 cm (or, in a few cases, 15-30, and 30-45 cm). Samples were taken at different positions (randomly selected) across each wheat field to account for variability in soil properties. For this study, only the surface soil sample (0-15 cm) was analyzed, which is a common depth to assess metal availability (Ding et al. 2013; Adam et al. 2004).

Grain from 65 wheat varieties were sampled from 18 locations over six years (Table 1.1). 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 in the field, while some were composited from grain bins. Grain variety and location was recorded for each sample. Prior to analysis, all grain samples were ground using a clean ceramic mortar and pestle.

A data analysis was conducted for all the data of soil and wheat in the database from 2014-2020. The sampling locations, wheat varieties, and the number of samples are presented in Table 1.1, Table 1.2, and Figure 1.1.

Table 1.1 Sampling years and locations.

Year	Sampled	Number of Samples	Location
2014	Soil	15	Aberdeen, Kimberly, Tetonia
	Grain	54	Aberdeen, Kimberly, Tetonia
2015	Soil	28	Ashton, Bonners Ferry, Genesee, Moscow, Nezperce, Moscow, Soda Springs, Tammany, Tensed
	Grain	239	Aberdeen, Ashton, Bonners Ferry, Genesee, Idaho Falls, Kimberly, Moscow, Nezperce, Moscow, Ririe, Rupert, Soda Springs, Tammany, Tensed
2016	Soil	28	Parma, Nezperce, Rexburg, Southwick, Tensed
	Grain	39	Parma, Nezperce, Rexburg, Southwick, Tensed
2018	Grain	09	Parma
	Soil	24	Parma
2019	Soil	104	Aberdeen, Soda Spring, Ashton, Kimberly, Rupert
	Grain	104	Aberdeen, Soda Spring, Ashton, Kimberly, Rupert
2020	Soil	24	Moscow, Tensed, Tammany
	Grain	24	Moscow, Tensed, Tammany

Table 1.2 Number of grain samples and sampling locations from 2014 to 2020 (total = 469).

Location	**Region**	**Number of grain samples**
Aberdeen	Southeastern	59
Ashton	Southeastern	31
Idaho Falls	Southeastern	29
Soda Spring	Southeastern	33
Rexburg	Southeastern	30
Ririe	Southeastern	05
Tetonia	Southeastern	15
Kimberly	South central	34
Rupert	South central	26
Parma	Southwestern	12
Moscow	North central	53
Tensed	North central	33
Tammany	North central	32
Southwick	North central	03
Genesee	North central	24
Nezperce	North central	26
Bonners Ferry	North	24

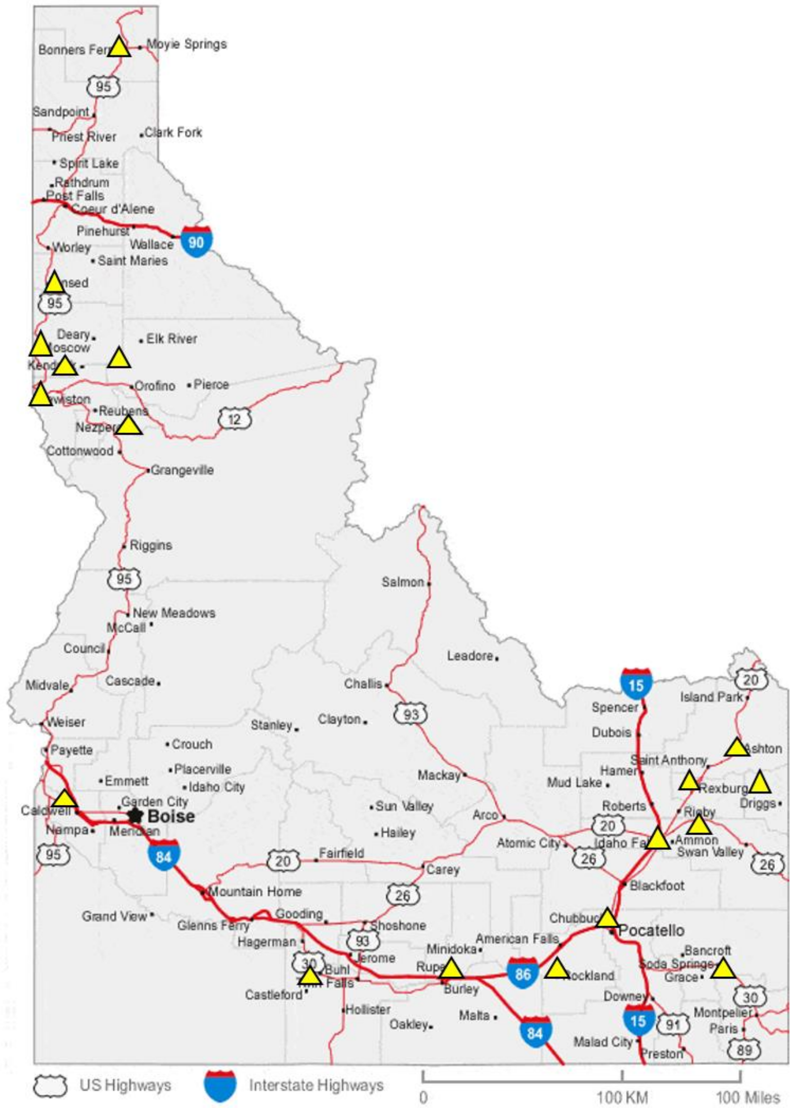


Figure 1.1 Grain sampling locations from 2014 to 2020

Chemical Analysis

Soil: 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 (1:1:1 HNO_3: HCl: H_2O), followed by analysis on an ICP-MS. (method detection limit (MDL) is 0.01 mg/kg). Soil pH was measured in a 1:1 soil: Deionized water paste.

Grain: The dried wheat grain was ground in a ceramic mortar and pestle and elemental concentrations in grain samples were determined by Bureau Veritas Inc. (Vancouver, BC). 1 g of ground grain samples were digested in HNO_3 and then aqua regia and analyzed by ICP-MS for ultralow detection limits (method detection limit is 0.01 mg/kg). Some grain had values below MDL and are reported as ½ MDL, which is 0.005 mg/kg.

Statistical Analysis

Descriptive statistics on the full dataset of soil and wheat grain Cd concentrations and soil properties were conducted. These included summary statistics such as the mean, median, standard deviation, interquartile range, etc. The database was filtered by different factors such as location, wheat variety, or wheat class, and the mean Cd concentration in soil was plotted to identify regions for optimum planting to produce low Cd grain. Subsets of wheat varieties were obtained from the database and the variations of Cd in grain concentrations in each variety were plotted using the bioaccumulation factor (Cd in grain/ Cd in soil) as an independent variable. Box whisker and bar charts were plotted of filtered data considering means and distributions of each filtered category. Only categories with at least four distinct observations are plotted. Box and whiskers are quartiles and outliers (4 times standard deviation), respectively. Error bars on bar charts are the standard error (SE). The filtering showed only a few varieties and locations were repeated with replicates, and thus hypothesis testing across the whole data set is not possible. Thus, data analysis approach uses the box whisker plots and SE on bar charts to make inferences between differences in grain Cd concentration in the filtered data. Soil pH and grain Cd concentration were plotted based on the sub-regions in Idaho. Correlations between elements in the soils and grain are evaluated using the Pearson correlation coefficient.

The bioaccumulation factor (BAF) for Cd uptake by each wheat sample was calculated by dividing the grain Cd concentration by the average soil Cd concentration at the site. The data were then filtered by variety, and the average and SE of BAF for varieties with at least five observations and sampled from at least three different sites were plotted. The filtered data were log transformed and ANOVA and Tukey mean separation test were conducted (OriginPro version 9.8.5.201). Based on mean separation results, data were grouped into either low, medium, or high BAF categories. By using the filtered data from each variety, the degrees of freedom are limited to this specific variety only, and thus variability is conservatively estimated; this reduces the chance of error in estimating differences in means from the filtered data. Furthermore, because the means are grouped into the three categories based on significant differences, the conclusions from this analysis are good to categorize the BAF of the varieties as low, medium or high BAF varieties, but cannot be used to identify significant differences between varieties.

Results and Discussion

Grain Cadmium Concentrations

Table 1.3 shows the metal concentration variation in the wheat grain in Idaho using the samples from 17 different locations. The variation of Cd accumulation in wheat grain is considerable and the maximum amount of Cd reported was 100 times greater than the minimum amount of Cd in grain. The variation is due to varying soil properties, climate, and wheat variety, as well as possible differences from agronomic practices (irrigation, fertilizer application rate and timing, tillage, etc.). The average level of Cd in wheat does not exceed the standard levels for the adult's food and they are safe for consumption by adults.

Table 1.3 Summary statistics of wheat grain metal concentration in Idaho (n=469)

	Zinc (mg/kg)	**Manganese (mg/kg)**	**Iron (mg/kg)**	**Cadmium (mg/kg)**	**Copper (mg/kg)**
Mean	25.6	43.1	5.00E-03	0.0670	4.01
Min	9.10	21.0	0.000	5.00E-03	1.71
Max	55.2	108	0.0170	0.280	6.82
Median	25.0	41.0	5.00E-03	0.0500	3.95
Standard error	0.375	0.657	7.10E-05	2.00E-03	0.0500
Inter quartile range (IQR)	11.0	16.0	2.00E-03	0.060	1.52

Figure 1.2 shows the Cd variation in the wheat grain in Idaho. The amount of Cd in grain varies from 0.005– 0.28 mg/kg. Most of Cd in wheat is between 0.02 to 0.08 mg/kg. According to the international food standards levels, a maximum amount of 0.2 mg/kg of Cd can be present in wheat grains. But this is limited to adult food. For infants, it is reported as 0.04 mg/kg. In this study almost all the samples are safe for adult consumption, and when the infant food limitations are considered, many of the grain samples have too much Cd. The industry target for baby food for wheat grain Cd amount is 0.03 mg/kg or less. This target is achieved in 144 samples out of 469 grain samples, indicating that ultra-low Cd wheat can be sourced from some Idaho wheat

growing regions if the optimum varieties are planted. A study conducted in Zhejiang province, China found that the mean values of Cd in wheat were 0.048 and 0.053 mg/kg in two different sites (Lu et al., 2020). The Cd concentrations varied from 0.01 to 0.10 mg/kg in wheat grain grown in agricultural non-polluted soils in Austria (Wenzel et al., 1996). In Manitoba-Canada, Cd in wheat grain ranged from 0.07–0.15 mg/kg dry weight (Gao and Grant, 2012). In a study in Brazil, the Cd concentrations of wheat grain ranged from 0.015 to 0.083 mg/kg, and they did not exceed the permissible level of 0.2 mg/kg (Corguinha et al., 2015). Therefore, Cd concentration varies by the variety, soil condition, climate, and anthropogenic activities.

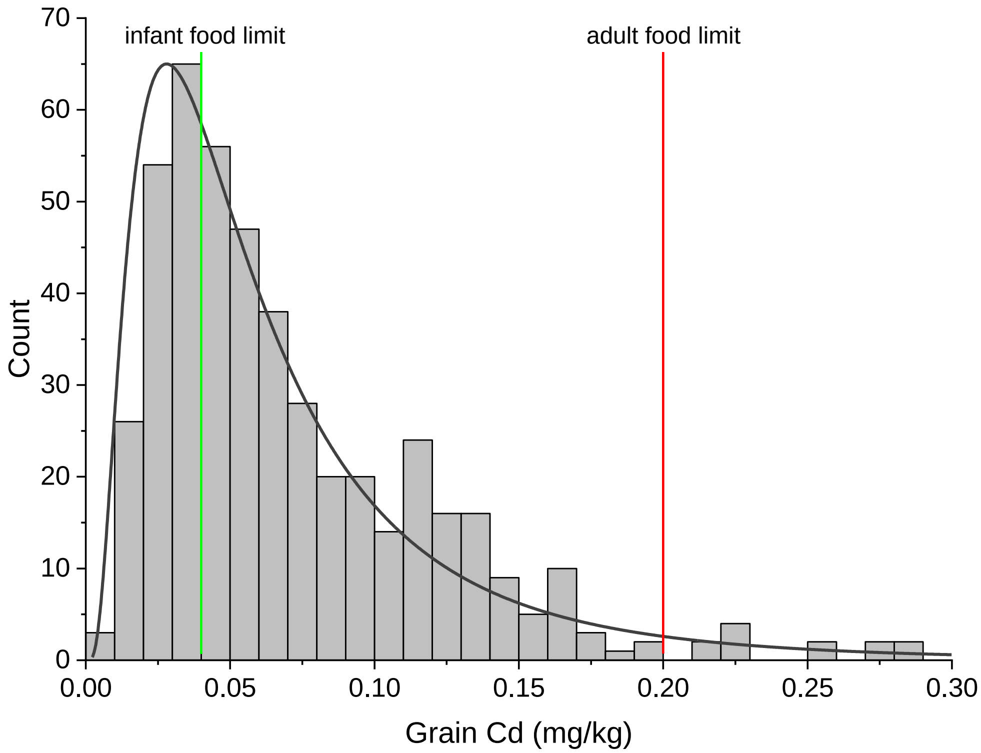


Figure 1.2 Grain Cd concentrations (n = 469). The red and green vertical lines represent the FAO’s maximum allowable Cd concentration in wheat for adults and infants, respectively. Distribution curve is log normal.

Wheat grain from Idaho Falls samples has the highest average Cd (Figure 1.3). Overall, the southeastern has the highest amount of Cd accumulation in grains. The lowest Cd contents in grain are in samples from sites in the north-central region; Tensed and Nezperce grain samples have the lowest Cd accumulation.

Figure 1.4 shows the Cd variation in selected wheat varieties of different wheat classes. UI Pettit has the highest amount of Cd in wheat grains (some samples exceeded the maximum allowable limit of Cd in adult food). Almost all wheat varieties have Cd concentrations greater than the infant food Cd limit for wheat grain. Most of the Cd in winter wheat varieties are between 0.01 – 0.11 mg/kg, while spring wheat varieties have 0.03-0.18 mg/kg. Guo et al. (2018) observed that four wheat cultivars out of 16 varieties in China had high Cd concentrations (Zhengmai7698, Zhengmai0856, Zhengmai366, and Pingan8), which were greater than the standard level (0.2 mg/kg). Spring wheat varieties in Idaho has the highest Cd accumulation compared to the other market varieties. These results are consistent with findings by Meyer et al. (1982) that winter wheat accumulated less Cd than spring wheat, but differ from Greger and Lofstedt's (2004) observations that grain Cd concentrations between spring and winter bread wheat were not significantly different. A confounding factor in comparing wheat classes in Idaho is that spring wheat is less commonly grown in northern Idaho where soil properties are dramatically different than southern Idaho, which affects Cd uptake.

Comparison of specific varieties by location indicates that Cd accumulation in wheat differs by soil properties and plant genomics. For instance, wheat varieties such as UI Platinum, CdDH-028, CdDH-266 grown in Soda Springs have different uptake of Cd than when they were grown in Ashton and Aberdeen (Figure 1.5). Cd concentration in the variety of UI Stone, which is commonly grown in Southeastern (SE), South-central (SC), and Southwest (SW) Idaho regions is highest in SE, then SC, followed by SW (Figure 1.6). UI Sparrow is a low Cd uptake variety (Figure 1.4), and was grown at four locations in Idaho (NC and SC Idaho regions). The lowest grain Cd concentration for UI Sparrow came from Tensed in NC, while the highest came from Rupert in SC Idaho. UI Stone was grown across all regions of Southern Idaho (Figure 1.4). Wheat grain Cd concentrations in UI Stone show a decreasing trend from SE, SC to SW Idaho regions. UI Silver was grown at several locations in SE and NC Idaho. Comparing the regional average Cd concentration in UI Stone shows a much lower concentration in UI Stone wheat grain grown in SW Idaho compared to SC Idaho (Figure 1.6).

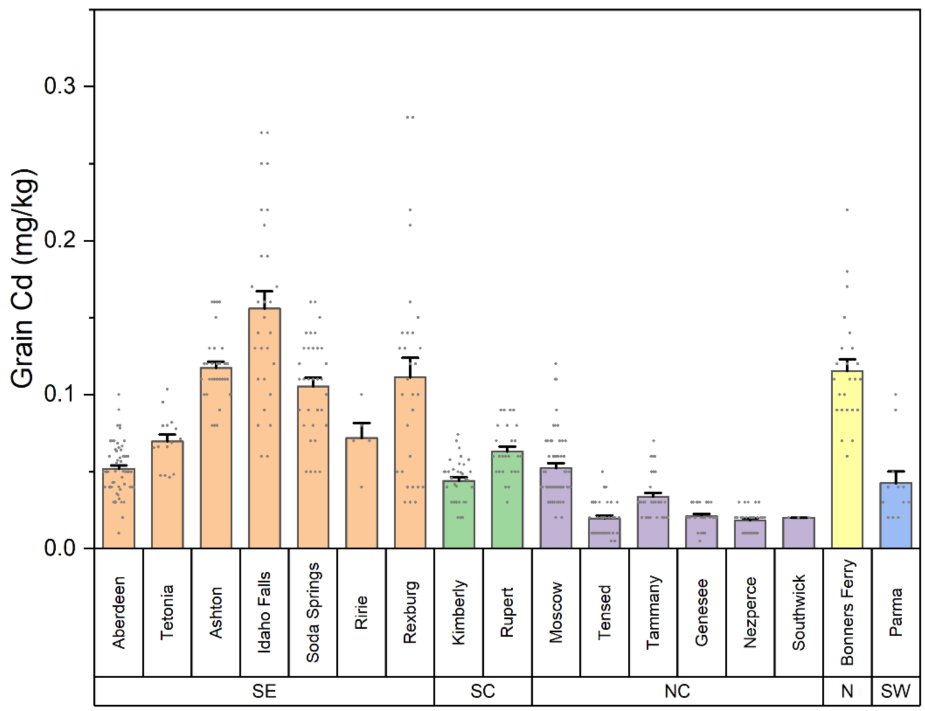


Figure 1.3 Average grain Cd in each sampling location. Error bars are standard error of mean.

Table 1.4 Mean concentrations of Cd in wheat grain for each location.

Location	Mean (mg/kg)	Std error	Location	Mean (mg/kg)	Std error
Aberdeen	0.0500	0.000	Nez Perce	0.0180	0.00200
Ashton	0.117	0.0130	Parma	0.0430	0.00800
Bonners Ferry	0.115	0.00800	Rexburg	0.111	0.0130
Genesee	0.0210	0.00200	Rupert	0.0630	0.00400
Kimberly	0.0440	0.0100	Soda Springs	0.105	0.0100
Tammany	0.0334	0.00227	Southwick	0.0200	0.000
Moscow	0.0520	0.00300	Tetonia	0.0700	0.00400
Tensed	0.0190	0.00200			

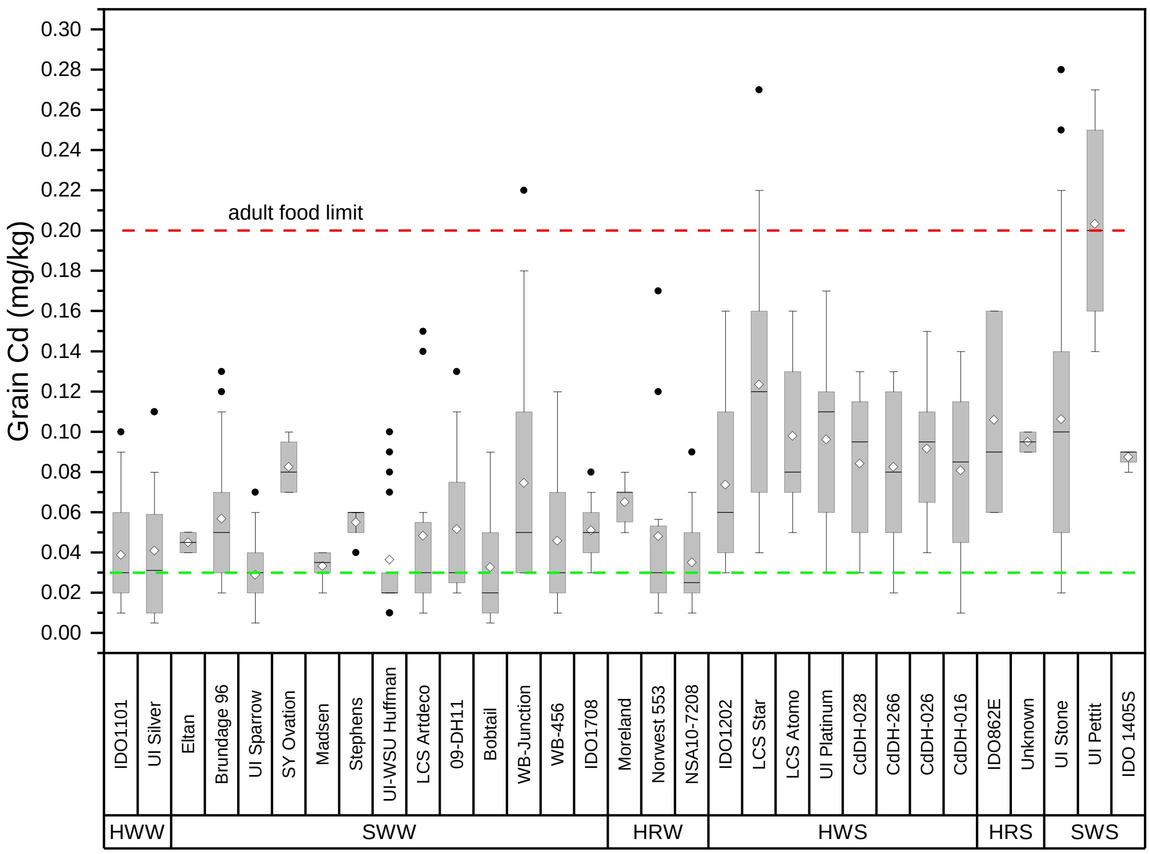


Figure 1.4 Grain Cd distribution by wheat variety and the class in Idaho. SWW and HWW are winter wheat varieties and SWS and HWS are summer wheat varieties.

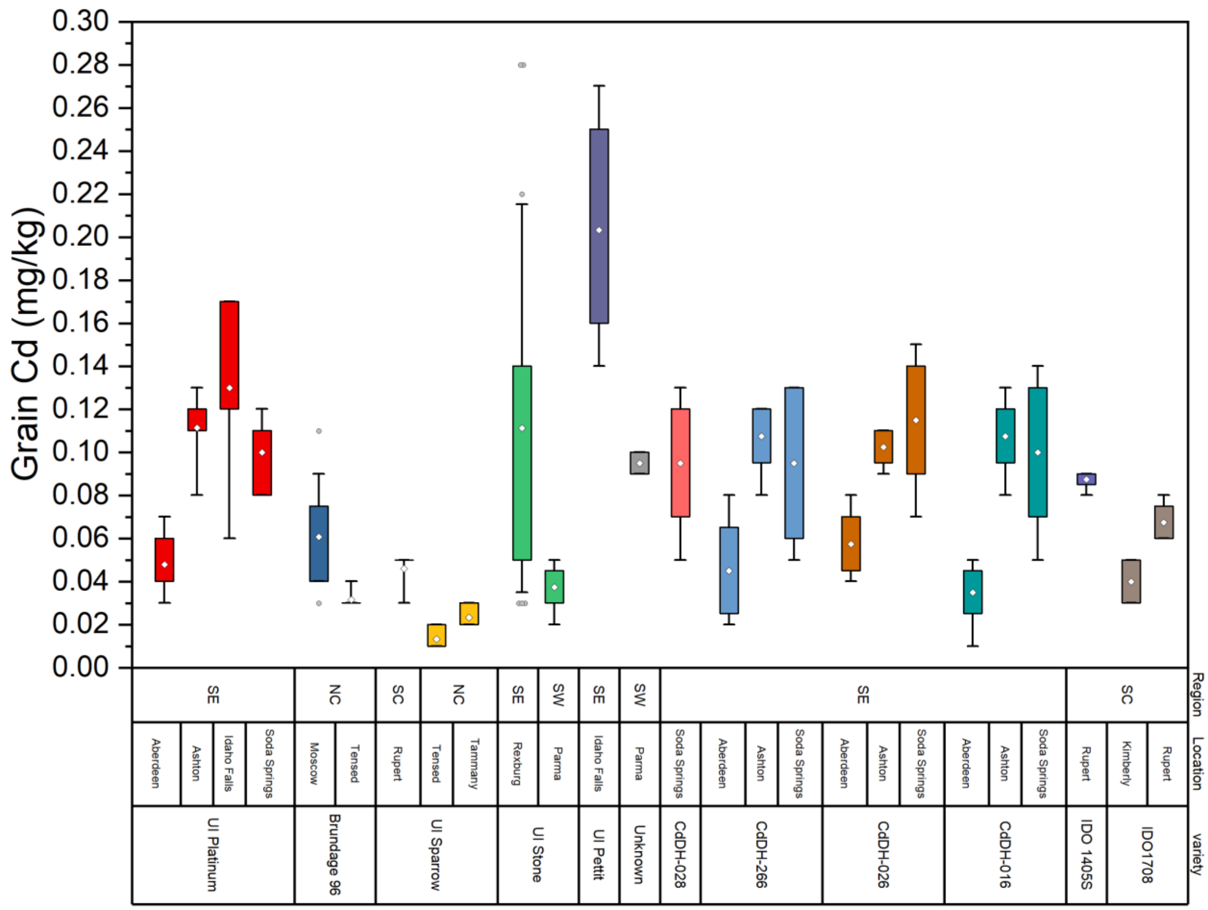


Figure 1.5 Box Whisker plot of Cd concentrations in grain filtered by location and region.

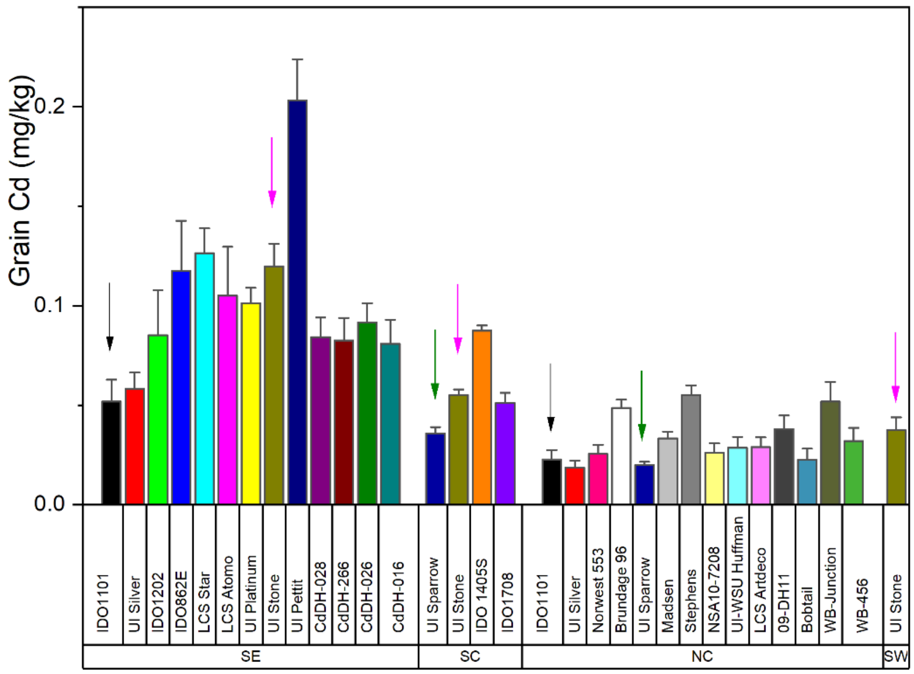


Figure 1.6 Mean Cd concentrations in different varieties grown in different regions. The arrows refer to the common varieties in different regions of SE, SC, NC, and SW.

Soil Cadmium Concentration

The average soil Cd in samples from Idaho samples collected in this study is 0.483 mg/kg and standard error is 0.010 (Table 1.5), exceeding the average Cd soil in the USA, which is 0.27 mg kg^{-1} (N=3045) (Holmgren et al., 1993). Holmgren et al. (1993), identified only a few regions with considerable natural enrichment in Cd, such as coast-ranges of central and southern California. Also, he stated that Cd concentrations in soils from the Western and North Central states are greater than those from North-Eastern and Southern states. According to a study by Page et al. (1987), the average Cd concentration in non-contaminated agricultural soil in the United States ranges from 0.1 to 1 mg/kg. The mean level of Cd concentrations in uncontaminated soil globally varies from 0.07 – 1.1 mg kg^{-1} (Kabata-Pendias and Pendias, 1984). In magmatic and sedimentary rocks, Cd typically does not exceed 0.3 mg kg^{-1} (Crook and Morrow, 1995). The background Cd levels in soils formed from igneous and metamorphic rocks are typically lower (0.02 to 0.2 mg kg^{-1}) than those formed from sedimentary rocks (0.1 to 25 mg kg^{-1}) (Crook and Morrow, 1995). Cadmium in most soils does not exceed 0.5 mg kg^{-1}, and higher values reflect anthropogenic inputs (Kabata and Pendias, 1985). Compared to the USA national soil Cd concentration average (0.27 mg/kg) (Holmgren et al., 1993), all the sub-region soils except north-central region's soil have greater concentrations of Cd (Figure 1.6). Southern Idaho soils contain more Cd than northern Idaho soils. The highest amount of total Cd reported in the southern Idaho soils (1.85 mg/kg) is more than twice the highest amount in northern Idaho soils (0.8 mg/kg). But most (~65%) of the soil Cd varied from 0.3 to 0.65 mg/kg in the south and 0.1 to 0.4 mg/kg in the north. Considering Cd content of sub-regions, southeastern Idaho soils have greater average Cd than south-central and southwestern regions (Figure 1.7). USGS statistics (2017) also showed that Cd in Idaho ranged from 0.2 to 6.0 mg/kg, and higher soil Cd concentrations occur in southern Idaho compared to northern Idaho soils. Compared with other PNW regions (Oregon, Washington, Montana) Cd in Idaho soil is little higher (USGS, 2017). The lowest Cd in soil within the United States occur in south central regions (Smith et al., 2007). Figure 1.8 shows the range of Cd variation in the soil in each site. Cadmium concentrations are lower in northern Idaho regions such as Tensed, Genesee, Moscow, Tammany, Parker Farm, and Nezperce compared to the southern Idaho sampling regions. Wilson et al. (2008) observed that average Cd in A horizons of West Virginia soils was 0.28 mg/kg, and it was 0.15 mg/kg in BC horizons. Other metal concentrations in the Idaho soils are typical soil levels (Table 1.5). However, the distribution of total Cd in soil (Figure 1.7) and grain (Figure 1.3) are similar; sub-regions with high Cd in the soil also had a high accumulation of Cd in wheat grains.

Table 1.5 Summary statistics of soil metal concentration in Idaho (n=227)

	Zinc (mg/kg)	**Manganese (mg/kg)**	**Iron (mg/kg)**	**Cadmium (mg/kg)**	**Copper (mg/kg)**
Mean	83.0	583	2.68	0.480	20.2
Min	37.5	96.0	1.08	0.0900	6.50
Max	128	2539	4.94	1.85	65.8
Median	83.7	604	2.52	0.450	20.4
Standard error	0.810	10.4	0.0400	0.0100	0.350
Inter quartile range (IQR)	19.2	266	1.01	0.250	10.3

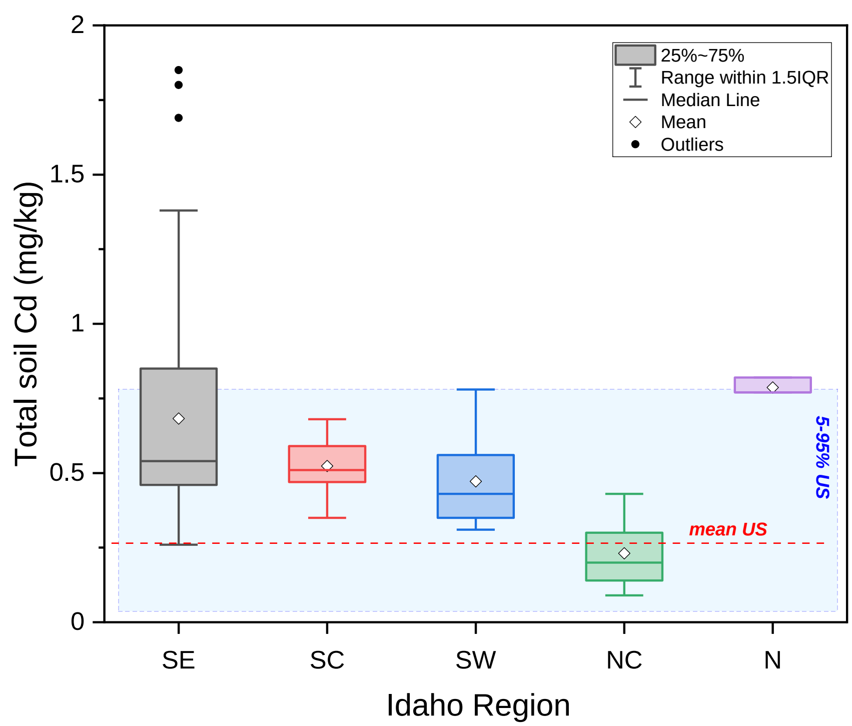


Figure 1.7 The total soil (0-15 cm) Cd distribution in different sub-regions in Idaho. SE, SC, SW, NC, and N represent the southeastern, south-central, south-west, north-central, and north regions. Mean and range are from Holmgren et al. (1993).

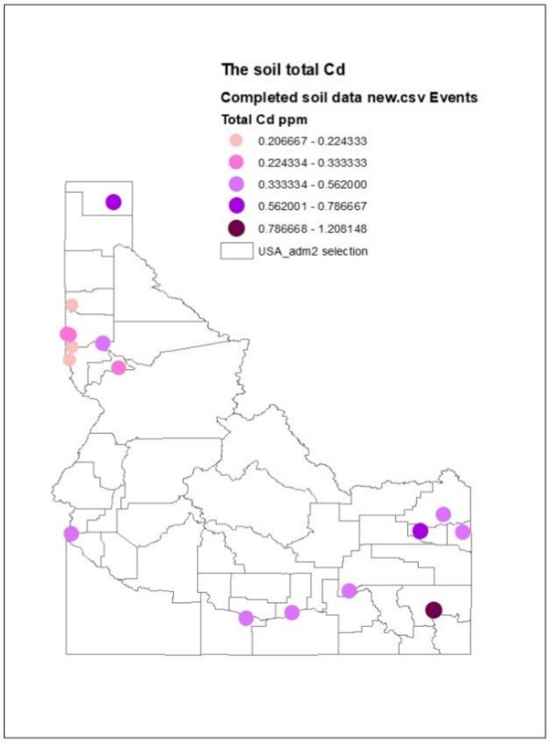


Figure 1.8 Geographical distribution of soil total Cd (0-15 cm) in 16 different sites in Idaho

Cadmium Bioaccumulation Factor

Bioaccumulation factor (BAF-Cd transfer coefficient) was calculated by dividing the grain Cd by the total soil Cd concentrations (Figure 1.9). BAF represents the proportion of Cd taken up in wheat grain relative to the amount in the soil, and thus is a plant variety specific availability index. It is used to assess the efficiency of wheat in metal accumulation and translocation. Soil Cd for each location was averaged to calculate BAF for the replicate wheat samples from each site, except for 2019 and 2020 samples, which have paired soil-grain samples. Bioaccumulation factor varies by wheat varieties from 0.05 to 0.7, except UI Pettit. UI Pettit has a higher BAF (0.9) than other varieties. However, it was only grown in Idaho Falls (Figure 1.5), so it is unclear if it is variety or soil factors that cause the greater grain Cd concentrations in UI Pettit. Wheat genotypes such as IDO1708, IDO 1101, UI Silver, UI Sparrow, Bobtail, and UI/WSU Huffman have very low BAF compared to UI Pettit.

Cadmium accumulation efficiency by plants is categorized into three groups according to their response to metal uptake. The first group is called excluders that maintain low metal concentrations in their tissues, up to a critical soil value. In contrast, accumulators, uptake metals regardless of the soil level (Bakar, 1981). Hyperaccumulators are extreme accumulators (Brooks, 1998). If the BAF < 1, plants are classified as excluders; if BAF values are in the range of 1-10, plants are accumulators; and if the BAF values >10, plants are hyper-accumulators (Dessalew et al., 2018, Zhao et al., 2003). Since the average BAF is less than 1 for all samples in this study, wheat is not an accumulator. Early researchers observed that both metal accumulation and metal tolerance by crops were species and cultivar-dependent (Fleming, 1965; Antonovics et al., 1971). Plants vary with the ability to absorb, accumulate, and tolerate metals within their tissues (Alloway, 1995).

Soil pH variation

Figure 1.10 shows the variation of pH in the surface soil samples in sub-regions in Idaho. Soil pH is a measure of acidity or alkalinity of soil. Soil pH can be lowered by organic acids exuded from soil biota and plant roots, or increased by the presence of carbonates in the soils. The highest average soil pH in Idaho occurs in the south-central sub-region and the lowest in north-central. A significant variation of pH within the soil occurs in southeastern soils, varying from acidic to alkaline pH values. Among the southeastern sampling locations Aberdeen soil had the highest average pH, which was around 8.4 and Soda Springs had the lowest average pH of 6. For all soils in north-central Idaho, pH values are less than 6, while in southern Idaho regions and northern Idaho soils, pH is more alkaline.

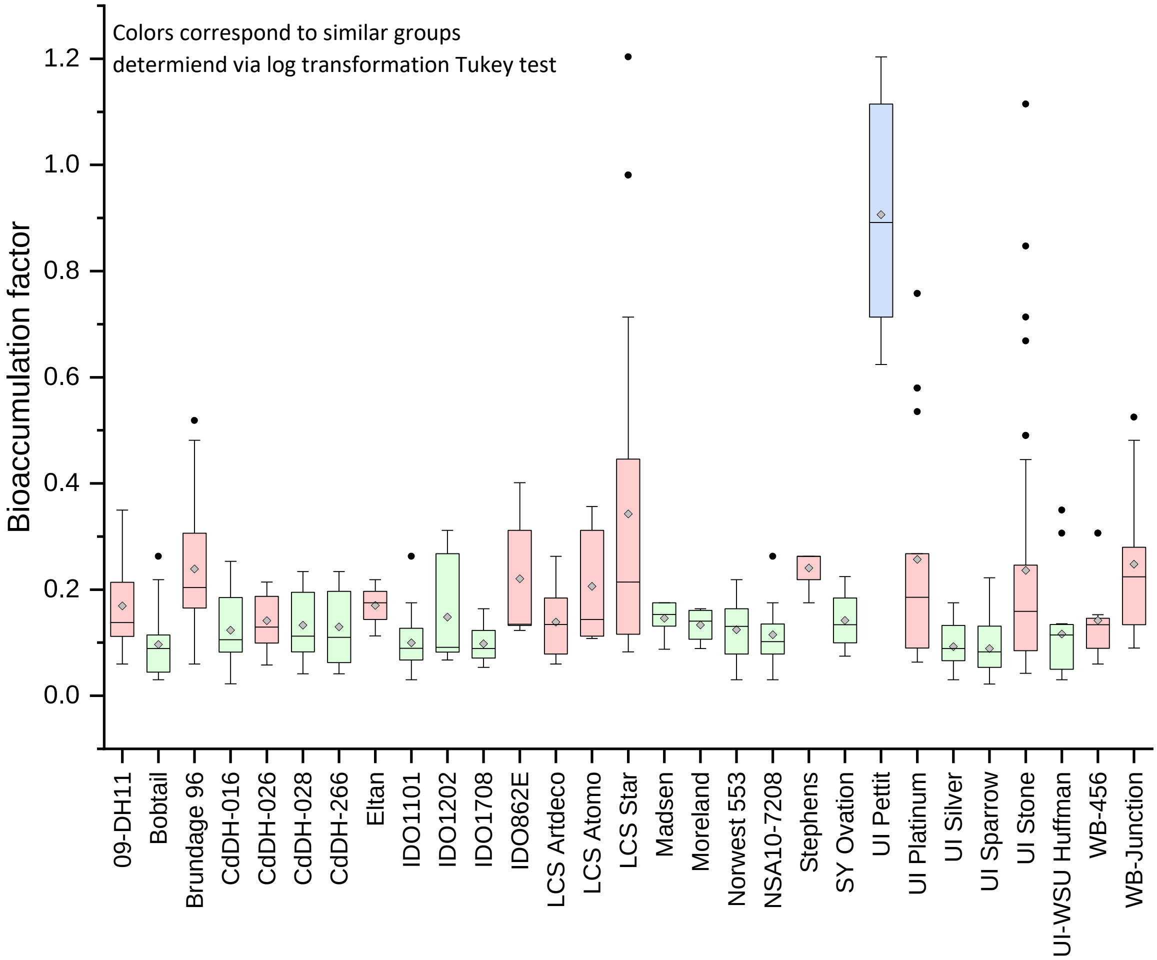


Figure 1.9 Cd bioaccumulation factor variation in grain from different wheat varieties. All means have at least five observations that were sampled from at least three different sites (except Stephens that was only grown at two sites). Samples are categorized into three groups based on ANOVA and Tukey mean separation test of log means. The green-shaded samples are not different than the lowest mean concentration variety (Bobtail). The red-shaded samples are different from the lowest mean concentration variety, and the blue-shaded sample is different than all other samples.

Harrison and Waites (1998) observed that soil pH above 6.5 promotes Cd mineral precipitation, which decreases Cd availability compared to adsorbed Cd that predominates processes at lower pH. The uptake of Cd is mainly regulated by free Cd^{2+} activity in soil solution. At low pH, dissolved Cd is higher, and Cd uptake by plants also increases with decreasing pH in soil (Speir et al, 2003). Oliver et al (1994) observed in South Australia soils grain Cd concentrations of wheat from eight long-term field trials decreased about 4-fold between pH 4.9 and pH 6.2. But they did not show any further trend at pH <4.9 and pH >6.2. Further, Gray et al. (1999) observed that Cd concentration was decreased in clover, lettuce, carrots, and ryegrass when soil pH increased above 5.5 to 7.0 in soil solution, although the magnitude of reduction varied between plant species and soil type.

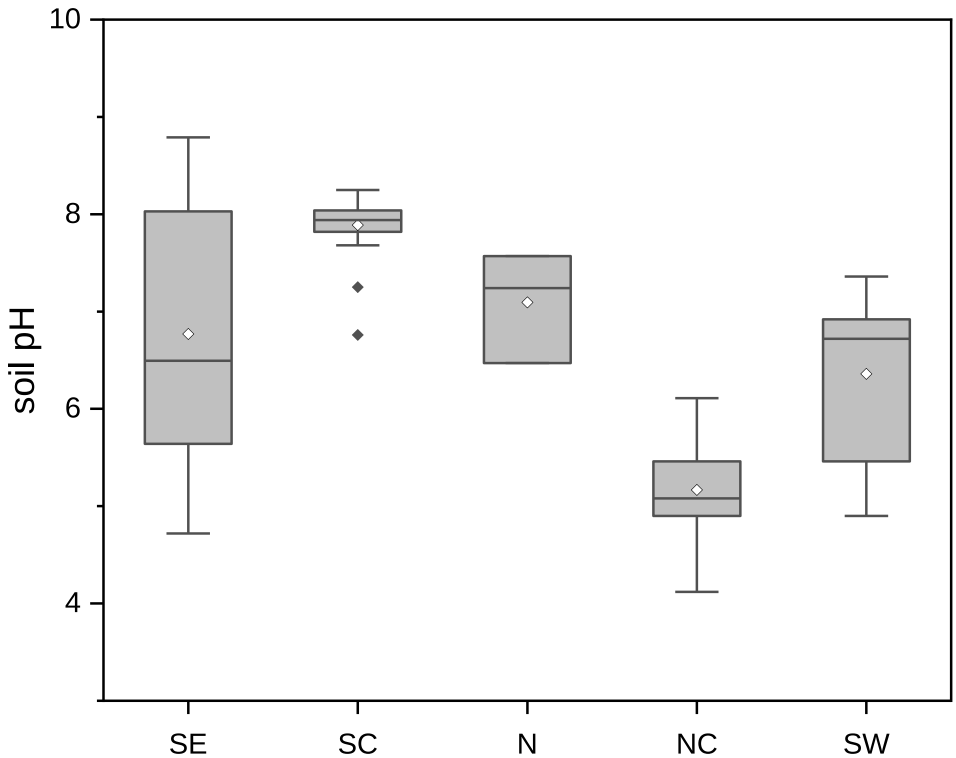


Figure 1.10 Soil pH variation in sub-regions in Idaho

Correlations of soil metals and grain metals

Pearson correlation analysis was applied to both soil and grain data separately to determine the relationship between soil and grain metal-metal correlations. Total Cd in soil is positively correlated with total Zn, total Cu, total Mn, and total P, and negatively correlated with total Fe (Figure 1.11). Abedi and Mojiri (2020) showed that increasing Fe, Mn oxide, and clay content in the soil may decrease Cd uptake by plants. Zimdahl and Skogerboe (1977) observed that hydrous Fe and Mn oxides may reduce concentrations of metal in soil solution by both precipitation and specific adsorption reactions. The correlation between Cd and Zn in the soil is moderately positive ($r = 0.499$). Total Fe and total Mn were negatively correlated with pH in the soil (r= -0.61 and -0.49 respectively). Other metal correlations are not significant with pH. In Turkey soils, Sungur et al. (2015) observed that pH and Cd were negatively correlated (r = -0.568).

For the grain Cd uptake, grain Fe, Mn, P, and Zn are positively correlated, and grain Cu is negatively correlated (Figure 1.12). But the correlations are very low, and not significant. Grain Cu and grain P are moderately positively correlated with grain Zn. Zinc is an essential micronutrient that may antagonize Cd uptake by plants because of their analogous properties (Rizwan et al., 2017). Oliver et al., (1994) observed that Cd concentration in wheat significantly decreased by Zn application and residual Zn concentration up to 5.0 kg Zn ha^{-1} in a study carried out in South Australia. Sadana and Singh (1987) observed that Cd uptake was drastically reduced by the application of a low dose of Zn in a pot study. Podar et al. (2004) suggested that human health risks from consuming plant parts grown on Cd-contaminated substrates is lower when Zn is also present in *Brassica juncea* because it reduces Cd uptake by 40%. Interactions between Cd and Zn can happen during plant uptake, transport within plants, and accumulation of Cd within edible tissues (Narwal et al., 1993 and Pence et al., 2000). But results of this study contradict those statements. Since the observations are very small in each variety and location, the interpretations are tentative because the data were not collected from a fully factorial experimental design. In addition to that, varieties used in this study were grown in different locations and their metal behavior can be different due to soil properties, climate, and other factors. Future research should explore the influence of different metals on Cd accumulation.

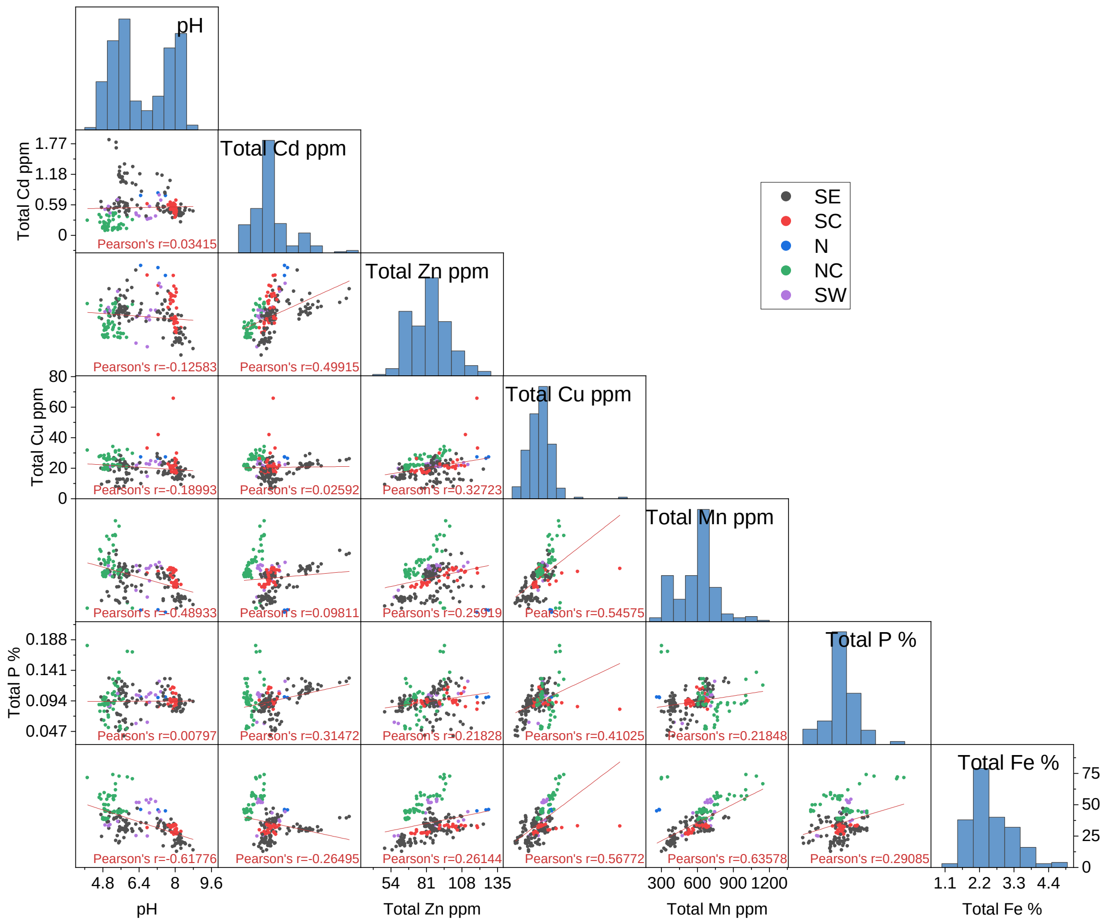


Figure 1.11 The soil metal and pH correlations in different sub-regions

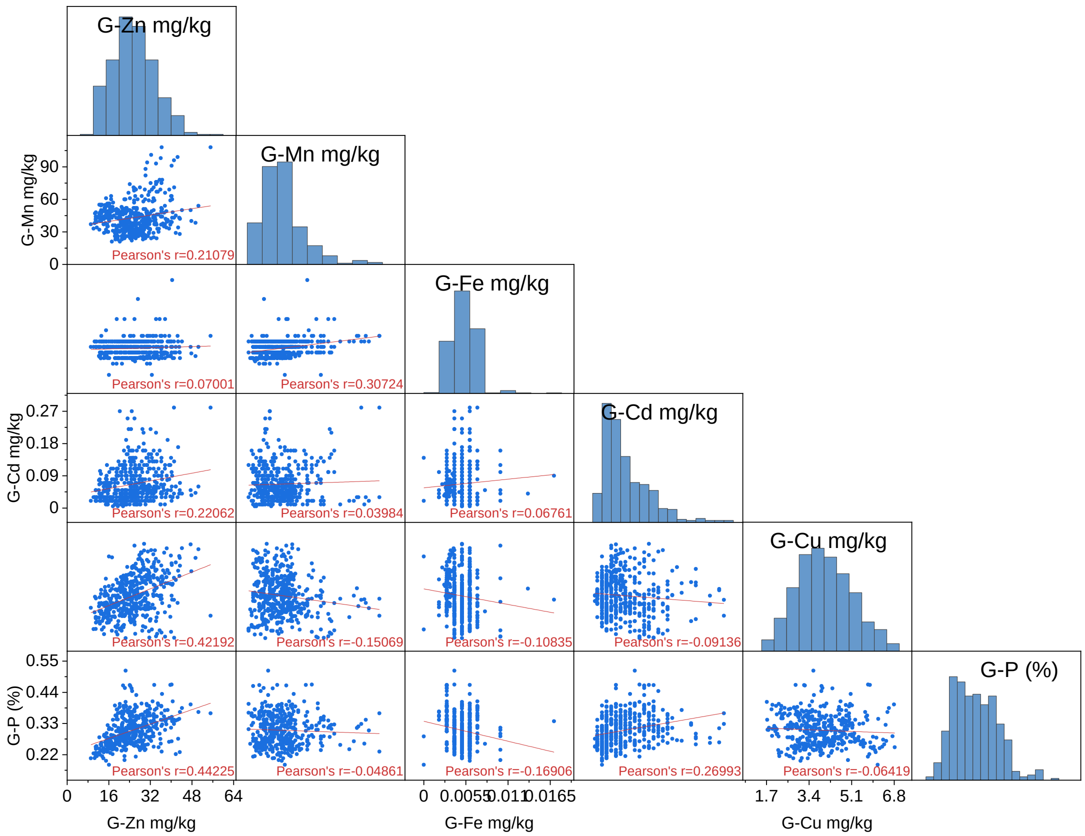


Figure 1.12 The grain metal correlation in wheat

Conclusion

This research suggests that Cd concentrations in wheat grains in Idaho varies by location, variety, wheat class, and soil properties. Overall, almost all wheat varieties in meets EFSA requirements for Cd concentration for adult consumption, but Cd concentration in most wheat grains exceed the EFSA maximum allowable limit of Cd in infant food. Cadmium concentration in soil was evaluated, and northern Idaho soils contained lower Cd than southern Idaho soils.

In this study, nearly 100 wheat varieties grown at different locations and different years were analyzed. UI Pettit has the highest average Cd level, while UI Sparrow has the lowest Cd uptake. Overall, Cd accumulations in spring wheat are greater than winter wheat varieties. Average bioaccumulation factor of all the selected wheat varieties is less than 1. Since sampling sites and strategies varied each year, future studies are needed to assess the Cd uptake by different wheat varieties in different locations.

Overall Recommendations for Sourcing Ultra low Cd gain

Table 1.6 Overall recommendation for the ultra-low Cd varieties by sub-region

Class	**Variety or breed line**	**Location**	
		Southern Idaho	**North Central Idaho**
Soft white wheat	UI Sparrow	Recommended	Recommended
	UI Brundage		Recommended
	IDO 1708	Recommended	
	UI Petit	Not Recommended	
	UI Stone	Recommended	
Hard white wheat	UI Platinum	Recommended	
	CdDH- 016, 026, 018, 266	Recommended	
	UI Silver	Recommended	Recommended

Table 1.7 Regions for wheat varieties to grow to achieve lowest grain Cd.

Region	**Variety or breed line**	**Recommendation**
South central	UI Sparrow, UI Stone, IDO 1708	Recommended
South eastern	UI Silver, IDO 1202, CdDH- 016, 026, 018, 266	Recommended
South western	UI Stone	Recommended
North central	UI Silver, UI Sparrow, UI Brundage, IDO 1101, Bobtail, Madsen	Recommended

Chapter 2: Effect of soil properties and variety on grain cadmium concentration in common wheat (*Triticum aestivum L.*)

Introduction

Cadmium is a non-biodegradable metal that naturally occurs in soils and is inherited from the soil parent material. Anthropogenic activities can also be a source of Cd enrichment in soils, including applications of fertilizers (Yang et al., 2014). Increasing food safety standards require that Cd in some foods must be reduced, including wheat grain. To achieve this goal, wheat varieties that uptake low amounts of Cd need to be determined, as well as regions that have soils with low amounts of available Cd to grow low Cd containing wheat.

The limitations and permissible levels in food commodities are reported under international and national food standards. Current regulations by the EPA are 0.001 mg/kg body weight per day of Cd intake from food (USEPA, 1999), which is the same value recommended by the FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2011)). Maximum concentrations in food products are established by the FAO Codex Alimentarius. The recommended maximum allowable Cd in wheat is 0.2 mg/kg (dry weight) for adults (EFSA 2014). For processed cereal-based food for infants and young children, maximum Cd levels allowable in wheat are set to 0.04 mg/kg (EFSA 2014). This marks a trend in awareness of Cd food safety risk and decreasing food safety standards, particularly for children and infants (Eklund and Oskarsson, 1999, Jean et al., 2018; Sanders et al., 2015; Taylor et al., 2014). So, the production of ultra-low Cd wheat grain is a necessity to minimize the health risk and improve the quality of food products.

Intake of Cd causes acute and chronic diseases such as fever, myalgia, chest pain, bronchospasm, hemoptysis, cardiovascular disease, lung and prostate cancers, and neurological disorders associated with chronic low dose Cd exposure (CSEM, 2008). Since food is a major pathway of Cd exposure, low Cd levels in food are needed to minimize the health risk.

An important factor in plant uptake is the speciation of Cd in the soil. Speciation refers to the chemical form (oxidation state or molecular composition and structure) in which elements occur. Depending on the amount and charge of Cd in the soil solution, there may be different physiochemical forms. Soluble Cd can be expressed as free hydrated cations (Cd^{2+}) or as complexes with organic or inorganic ligands (e.g., $CdCl^{+}$). Cadmium can also be associated with organic and inorganic colloids. Although colloids are not usually considered part of soil solution, their close association with the solvent affects Cd behavior (Helmke, 1999). The association of Cd with these fractions affects Cd phytoavailability (Jafarnejadi et al., 2011). Yang et al. (2014) observed that the main forms of Cd in soils and sediments are the exchangeable fraction. In contrast, Chlopecka (1996) found that the fixed Cd fraction in soil was the most abundant, and the concentrations of exchangeable Cd were relatively low.

Phosphate fertilizer and fossil fuel combustion have been identified as major sources of Cd contamination to agricultural soils, contributing 41% and 22% respectively to the Cd taken in by humans from industrial processes (Van Assche, 1998). Phosphate fertilizers contain elevated Cd due to the occurrences of Cd in the sedimentary rocks mined to source P, and some of the Cd is conserved in the processing and manufacturing process (Erdem et al., 1996). On average, phosphate rock contains 25 mg kg^{-1} of Cd (Mortvedt and Beaton, 1995). Phosphate rock deposits in Idaho range from 40 to 150 mg Cd/kg rock with an average 92 mg/kg Cd, which is the highest average Cd in phosphate rock used for fertilizer in the world (Roberts, 2014). The amount of Cd transferred from rock to fertilizer depends on fertilizer manufacturing process. In single superphosphate and triple superphosphate manufacturing processes, nearly all of the cadmium transfers to the fertilizer (Roberts, 2014). In wet process phosphoric acid processing, about 55 to 90 % of the cadmium transfers to the fertilizer (Roberts, 2014). Thus, long-term use of phosphorus fertilizer may increase Cd in surface soils (Page et al., 1987; PPRC, 2017), and subsequently, Cd is present in nearly all agricultural soils.

Cadmium accumulation levels in plants are strongly influenced by soil properties and plant variety (Mench et al., 1997; Gramlich et al., 2017), and natural variation in Cd accumulation levels within plant species can be exploited to breed low Cd-accumulation wheat cultivars (Grant et al., 2008). Many countries have established soil quality standards based on the total metal content in the soil. Predicting risk using only total Cd concentration in soil may not be accurate, and it is important to measure or predict bioavailability to improve risk assessment and management strategies (Ding et al., 2013). Metal uptake by plants is dependent on bioavailability, which may vary in soil with different soil properties and conditions that influence the partitioning of metals between soil solid and solution (Rieuwerts et al., 1998).

To avoid pollution in the food chain by Cd, it is essential to assess its bioavailability in soil. To predict Cd bioavailability, McBride (2002) used soil pH and total soil Cd to develop an empirical equation that predicted Cd bioavailability for plant uptake from sewage sludge treated soils:

$$\text{Log (Cd)}_{\text{crop}} = \text{a} + \text{b log (Soil Cd)} - \text{c (Soil pH)}$$

where, a, b, c coefficients varied, depending on the particular soil, climate, and crop species. As an example, this equation was applied to determine Cd uptake by Swiss Chard and predicted as $\log(\text{Cd})_{\text{chard}} = 4.36 + 0.673 \log(\text{Cd})_{\text{T}} - 0.667$ pH ($R = 0.897$). This relationship proved accurate for the comparison of Cd bioavailability in sewage-sludge-treated and non-contaminated soil (McBride, 2002). Page et al. (1981) found that increasing soil pH is one of the most recommended management practices to minimize Cd uptake by wheat because of the influence of pH on the ionic speciation in soil solution and pH-dependent Cd adsorption. Oliver et al. (1998) observed smaller decreases in grain Cd concentration with increasing soil pH. Hattori et al. (2006) reported the effect of soil acidification on Cd uptake differed among the plant species, and a soil pH 3.5 led to an increase in Cd uptake by Kenaf plant and decreased Cd uptake by sorghum plant because of the root damage due to Cd toxicity.

Many studies have found that different soil properties influence Cd accumulation in different crops and grain. As soil factors, pH, CEC, organic carbon, bioavailable metals, soil total metals, Cl, S, Ca, Mg, Zn and Mn have been identified as important factors affecting Cd uptake by plants. But the influence of these factors differs by site, variety, type of crops, and availability of other nutrients antagonistic or synergistic with Cd uptake (Grant et al., 2013). Increasing Cl^- affects Cd uptake by plants, largely by mobilizing soil Cd as Cd-Cl complexes that increase Cd availability for plant uptake (Dahlin et al., 2016). The reduction of sulfate into sulfide and formation of CdS reduces bioavailable Cd in soil solution and minimizes Cd uptake by plants (Furuya et al., 2016). Soils with high organic carbon decrease the Cd availability for plant uptake (Liu et al., 2015) because organic carbon can protect plants from heavy metal toxicity due to chelation involving organic acids and heavy metal ions (Wang et al., 2013).

Several studies have developed empirical models to predict Cd concentration in plants based on soil properties (Table 2.1) (Ding et al., 2013, Liu et al., 2015, Wen et al., 2019). Soil pH and soil total Cd have been identified by many studies as significant factors for plant tissue Cd concentration. To develop the best model for grain Cd concentration, stepwise regression analysis has been widely used (Ding et al., 2013, Liu et al., 2015, Wen et al., 2019). Ran et al. (2016) used principal component analysis (PCA) to simplify the complexity of relations between soil properties and address multi-collinearity problems. Baize et al. (2009) used the results from canonical correlation analysis before employing a stepwise linear regression because canonical correlation analysis can be used to identify the most strongly correlated linear combinations of the variables. Ding et al. (2013) showed that the inclusion of more soil properties in the model improves the correlation performance between Cd in plants and soil compared to the models with only total Cd concentration.

Table 2.1 Models for predicting Cd concentration in plants.

Crop/ plant	**Site**	**Soil condition**	**Model**	**Adj. R^2**	**Reference**
Carrot	Soil samples from the surface of farmlands throughout China	Cd was added	$\log(Cd_{carrot})$ = = 1.30 − 0.24pH + 1.27 $\log[Cd_{soil}]$ − 0.40 log[OC]	0.90	Ding et al., 2013
Wheat plant	Soil samples from highly polluted south China and less polluted north China	Polluted	Log BAF =0.279 pH + 1.386 *BAF = Bioaccumulation factor	0.85	Liu et al., 2015
Rice grain	Soil samples from the highest Cd background area in China	Polluted	Log[BAF]= −0.683 × log [Soil Ca] − 0.161 × pH − 0.237	0.48	Wen et al., 2019
Wheat grain	Soils from the major areas of Austria. Cd level of soil below than 0.4 mg/kg	Agricultural with typical Cd levels	Cd_{grain} = -0.0958+Cultivar + 0.277Cd_{soil} + 0.019 pH – 0.022 OC + 0.0004 Cl^- - 11.4 Ca_{soil}	0.90	Wenzel et al., 1996
Wheat grain	Soil samples from Britain	Soil with sewage sludge	$\log10(Cd_{grain})$ = 0.28 + 0.44 $\log10(Cd_{total})$ − 0.18 pH	0.49	Adams et al., 2004
Wheat grain	Soils from Netherland	Agricultural with typical Cd levels	$\log10(Cd_{grain})$ = 1.022 + 0.749 $\log10(Cd_{total})$ − 0.257 pH − 0.277 log10(SOM)	NA	Brus et al., 2005
Wheat grain	Contaminated soil from	Polluted	$\log10(Cd_{grain})$ = 0.703 + 1.04 $\log10(Cd_{total})$ − 0.175 pH	0.61	Ran et al., 2016

	industrialization in China				
Wheat grain	Northern France.	Agricultural with typical Cd levels	Cd_{grain} = – 1.21 – 0.011*$CaCO_3$ – 0.173*Mn_{total} + 0.421*Cd_{DTPA} – 0.145*Zn_{DTPA} + 0.214*Cd_{NH4NO3} – 0.166*Fine sand + 0.111*Coarse sand	0.50	Baize et al., 2009

Plant uptake depends on root structure, plant physiology, and plant biochemistry, which can vary across varieties of a particular plant. The accumulation of heavy metals in plants occurs both in roots and above-ground tissue (Rattan et al, 2005). Plant factors that affect metal uptake are: physical processes such as root intrusion, water, and ion fluxes and their relationship to the kinetics of metal solubility in soils; biological parameters, including kinetics of membrane transport, ion interactions, and metabolic fate of absorbed ions; and the ability of plants to adapt metabolically to changing metal stresses in the environment (Cataldo and Wildung, 1978). Sadana and Singh, (1987) suggested that applied Cd is readily absorbed by wheat and easily translocates from roots to above-ground plant parts because more than 10-fold increase in the Cd concentration by wheat was recorded by applying 10 mg/kg of Cd into the soil, compared with the no-Cd control treatment. Compared to other metals, Cd has a higher potential to accumulate in edible parts of plants and shoots, thus posing a risk to animals and humans that consume the plants (Yang et al, 2014). However, the Cd content is generally greater in roots than in the above-ground tissues because the roots act as a barrier to the uptake and translocation of the Cd and its accumulation can be ordered as, roots>stems>leaves>fruits>seeds (Bulum, 1997). Within wheat grain, Cd is distributed through the endosperm and bran (Guttieri et al., 2015).

Cadmium forms complexes with ligands, and those complexes contain several amino acids with glutamic acid, cysteine and glycine as the major constituents to detoxify the heavy metals in plants (Hasan et al, 2009). Enzymatic activities or compound contents in the antioxidant system and glutathione-ascorbic acid cycle are increased significantly (Li et al, 2018). Cadmium also has a higher affinity with thiol group in enzymes and other proteins that creates plant toxicity (Sadana and Singh, 1987). The major Cd toxicity mechanism is identified as excessive production of reactive oxygen species (ROS) in plants (Gajewska and Sklodowska, 2010). But, according to Li et al. (2018) the oxidation of ROS in turnip remains stable with the Cd stress due to the maintenance of ROS hemostasis and osmotic adjustment by antioxidant system to maintain the stability of osmotic potential.

Research Goal

The goal of this study is to determine the most important soil factors for predicting Cd concentration in wheat grain growing in Idaho, USA, and how varieties respond to different soil properties. Wheat is grown in northern and southern Idaho. In northern Idaho winter wheat is grown in under rainfed condition, while in southern Idaho winter and spring wheat are grown, and many locations are irrigated. Soils throughout Idaho are highly varied. In this study we sampled wheat grain and soils from three locations in southern Idaho and three in northern Idaho. Metal concentrations, nutrient availability, and soil physicochemical properties were measured in paired soil-wheat samples collected from the sites. Machine learning algorithms were used to do multi-variate modeling and evaluate which soil properties are most important for predicting grain Cd concentration.

Methods

Sampling

Grain and soil samples were collected from three sites located in northern Idaho (Moscow, Tammany, and Tensed) and southern Idaho (Ashton, Aberdeen, Soda Springs). Grain varieties were planted in a randomized complete block design with four replicates at each location. Each plot was 1.5×3 m.

Table 2.2 Number of genotypes* planted at each location.

Location	UI Platinum (Parent)	LCS Star (Parent)	Plt-Star cross	UI Sparrow	Brundage	Total number of lines
Ashton	1	1	4			6
Soda Springs	1	1	4			6
Aberdeen	1	1	4			6
Moscow				1	1	2
Tensed				1	1	2
Tammany				1	1	2

Hard white spring wheat was planted at Ashton, Soda Springs, and Aberdeen in southern Idaho: UI Platinum, LCS star, and four lines from UI Platinum× LCS Star cross variety (CDdH-016, -018, -026, and -266). Soda Springs is dryland and Ashton and Aberdeen are irrigated. In total, 24 samples were collected from each location at the three southern Idaho sites, making a total of 72 samples. Two soft white winter wheat varieties were planted at Moscow, Tensed, and Tammany in northern Idaho and eight samples from each location were collected for a total of 24 samples from northern Idaho plots. The wheat varieties tested in this study are common wheat varieties grown in Idaho and are varieties that have shown high and low Cd uptake (See Chapter 1); LCS Star and Brundage are generally observed to have higher grain Cd concentrations than UI Platinum and UI Sparrow.

A paired soil sample was collected from each plot at each site. To collect the soil sample, 3-cm diameter by 15-cm long cores of soil were taken and separated for the corresponding depths of 0-15, 15-30, and 30-45 cm. Samples from each depth at each plot were mixed thoroughly in a bucket. The mixed soil samples were placed into a sampling bag and labeled. For this study, only the 0-15 cm samples were analyzed.

Soil Analysis

The top 15 cm soil samples were air-dried and sieved through a 2-mm sieve. The sieved samples were sent to the Bureau Veritas Inc. (Vancouver, BC), an ISO/IEC 17025 accredited laboratory to measure the total elemental concentrations using three-acid digestion method: 0.25 g of soil sample was heated in HNO_3, $HClO_4$, and HF to fuming and taken to dryness, followed by dissolution in HCl and analyzed by ICP-MS (method detection limit is 0.01 mg/kg). The pH and EC in the soil solution were analyzed using the 1:1 soil to DI water method. Total N and C were determined via dry combustion using a Vario Max CNS analyzer (Elementar Americas, Inc., Mt Laurel, NJ). To determine the organic matter content in the soil, the loss on ignition method was used. After initial oven drying at 105 ºC, 0.5 g of samples were ignited in a muffle furnace for 2 hours at 360 ºC. The percent weight loss during the ignition step was reported as OM-LOI (Nelson and Sommers, 1996). Cation exchange capacity of soil was measured in 1:25 solid to solution ratio in 1 N NH_4OAC (Chapman, 1965). The clay content was analyzed using 1:4 soil:dispersing solution by the hydrometric method (Bouyoucos, 1962). The chloride content in soil was measured using saturated 1:10 soil:solution extract and analyzed using ion chromatography (Chemical Test procedure, 2005). To analyze the ammonium and nitrate in soil extract, 2 M KCl extraction (10:1 extractant: soil) method was used as described by Keeney and Nelson (1982). Available P in soil was extracted by using 1:20 soil:sodium bicarbonate (0.5 M $NaHCO_3$) solution and analyzed by ascorbic acid method (Oslen et al., 1954). To determine the available K in the soil, 1:5 soil:solution of 0.5M ammonium acetate solution was used and analyzed using flame emission spectroscopy. (Hendershot et al., 1993). To determine the DTPA metal concentrations, 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 492 G for 10 minutes, filtered through 0.22 μm polyethersulfone (PES) membrane filters, and extracts were analyzed on an ICP-OES (Agilent 5110) for Cd, Cu, Fe, Mn, and Zn standardized using NIST traceable standards (MDL = 0.004 mg/L).

Wheat Grain Analysis

The dried wheat grain (whole grain separated by a small winnower) was ground in a ceramic mortar and pestle and elemental concentrations in grain samples were determined by Bureau Veritas Inc. (Vancouver, BC) as follows: 1 g of grain samples were digested in HNO_3 and then aqua regia (1:1:1 HNO_3: HCl: H_2O) and analyzed by ICP-MS for ultralow detection limits (method detection limit is 0.01 mg/kg dry weight).

Figure 2.1 Ground wheat grain sample

Statistical Analysis

All analyses were conducted in R v 4.0 (R Development Core Team, 2019). Data were analyzed using one-way ANOVA mixed effect models (Midway, 2021), and a mean separation test using Tukey HSD (α=0.05) model to determine significant differences. Box and Whisker plots are plotted to identify the Cd variation in soil and grain by location or variety. In Box and Whisker plot, the bold line represents the mean, boxes are the upper and lower quartiles, and whiskers indicate the variability outside the upper and lower quartiles. Points are the outliers. Multicollinearity among soil variables was examined by computing pairwise Pearson correlations and conducting principal component analysis (PCA) of the variables using a correlation matrix. The influence of each soil variable on grain Cd was initially estimated using multiple linear regression using the package 'StepReg' (Li et al., 2020) and one-way ANOVA was performed to identify significance of the variables to grain Cd concentration. A bidirectional stepwise selection was conducted to narrow the number of explanatory variables. The entry and exit criteria used a p-value cut-off of 0.10 for a single variable's slope and the model was optimized using adjusted r-squared and Mallows' Cp. Random Forest was also used to predict grain Cd from soil properties. The 'randomForest' package in R was used (CRAN, 2018), setting the number of trees at 500, the node size at one, the number of observations in each tree as the total number of observations in the data set after sampling with replacement, and the number of variables to include in each split at 9 (Liaw and Weiner, 2002). The 'pls' package in R was used in Partial Least Square (PLS) modeling (Mevik & Wehrens, 2020), which is used as a multivariate regression model to predict the Cd concentration using significant soil factors.

Results

Relationship between variety and location to the grain Cd concentration

From the southern Idaho sites, grain Cd concentration in Aberdeen samples is approximately half the grain Cd concentration in Ashton and Soda Springs (Table 2.3 and Figure 2.3). At Soda Springs, LCS Star has the highest grain Cd concentration.

Table 2.3 Grain Cd concentrations (mg/kg) of different varieties in each sampling site

Variety	Location												Variety	
	Aberdeen		Soda springs		Ashton		Moscow		Tammany		Tensed			
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	mean	SE
UI Platinum	0.050	0.010	0.098	0.010	0.11	0.00							0.086	0.0088
LCS Star	0.058	0.0050	0.13	0.014	0.12	0.0025							0.10	0.010
CdDH-016	0.035	0.010	0.10	0.020	0.11	0.010							0.081	0.012
CdDH-026	0.058	0.010	0.12	0.017	0.12	0.017							0.096	0.011
CdDH-028	0.048	0.010	0.095	0.017	0.11	0.011							0.083	0.010
CdDH-266	0.045	0.013	0.095	0.021	0.11	0.010							0.084	0.012
UI Sparrow							0.028	0.0025	0.025	0.0029	0.015	0.0029	0.023	0.0022
UI Brundage							0.073	0.0025	0.063	0.0025	0.033	0.0025	0.056	0.0053
Location	0.049	0.0036	0.11	0.0066	0.11	0.0034	0.050	0.0087	0.044	0.0073	0.024	0.0038		

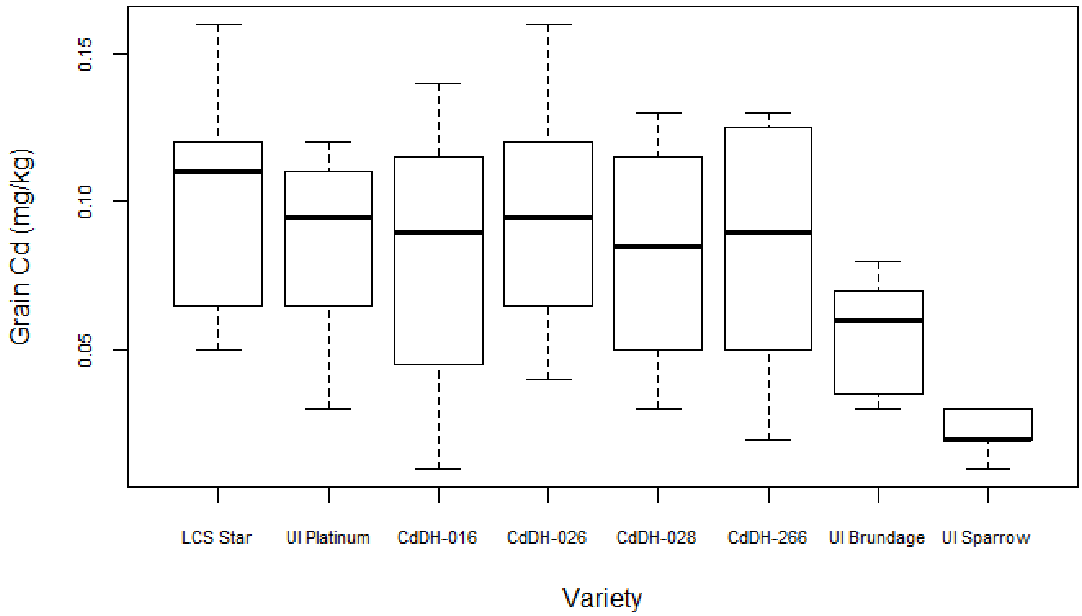


Figure 2.2 Box whisker plots of grain Cd in different wheat varieties of LCS Star, UI Platinum, CdDH-016, 026,028, 266, UI Brundage, and UI Sparrow.

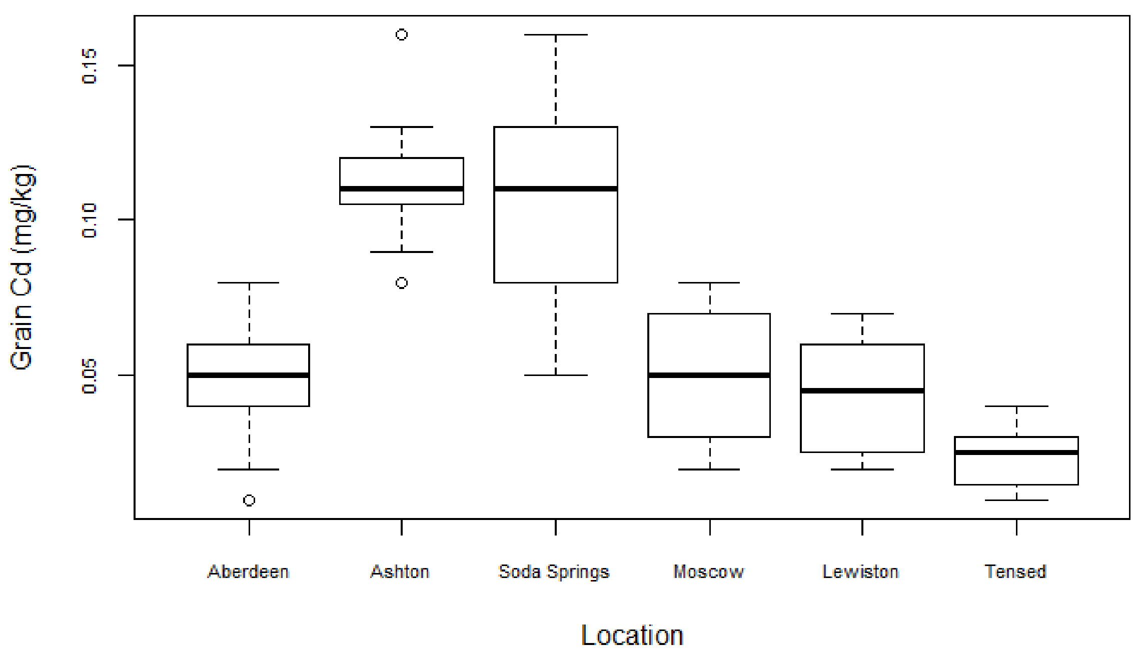


Figure 2.3 Box whisker plots of grain Cd distribution in different sampling locations

A mixed-effect model was used to identify the relationship between variety and location for the grain Cd concentration in each southern and northern Idaho regions separately. Table 2.4 shows the summary ANOVA table. For the southern Idaho data, location is a significant factor in grain Cd concentration, and variety is not a significant factor. There are no interaction effects between variety and location in the southern Idaho region for all lines of wheat (including hybrids); however, excluding hybrids in the ANOVA showed that Cd concentration in the two parent varieties are different (Appendix Table 2.21). Grain Cd concentrations in LCS Star are significantly less than UI Platinum. In the northern Idaho region both main effects (location and variety) and their interaction are significant for the Cd concentration (Table 2.5).

Table 2.4 Type III analysis of variance table for the effect of variety and location to the grain Cd concentrations in southern Idaho

	Sum of squared	**Mean squared**	**Degree of freedom**	**F value**	**Pr(>F)**
Location	0.01334	0.006671	2	17.70	0.003045
Variety	0.003807	0.0007614	5	2.020	0.09392
Location×Variety	0.001514	0.0001514	10	0.4016	0.9389

Table 2.5 Type III analysis of variance table for the effect of variety and location to the grain Cd concentration in northern Idaho

	Sum of squared	**Mean squared**	**Degree of freedom**	**F value**	**Pr(>F)**
Location	0.001114	0.0005571	2	40.11	< 0.001
Variety	0.006667	0.006667	1	480.0	< 0.001
Location×Variety	0.0008083	0.0004042	2	29.10	< 0.001

A Tukey HSD test was conducted to analyze the pairwise comparison between variety-variety in different locations and location-location comparison in different varieties. Confidence intervals were created for all pairwise differences between all factor level means while controlling the family error rate to a level of 0.95. The difference between Brundage and UI Sparrow varieties in both Tammany and Moscow are significantly higher than Tensed

(Appendix Table 2.13, Figure 2.4). When considering the location comparisons, Tammany-Tensed and Moscow-Tensed are significantly different for Brundage, but, for UI Sparrow, only the Moscow-Tensed locations are significantly different ($p<0.05$) (Appendix Table 2.13).

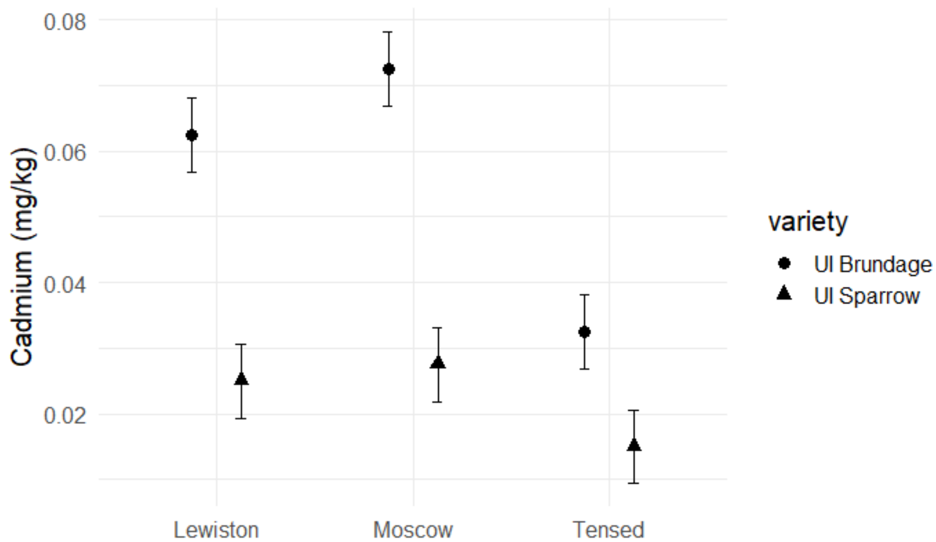
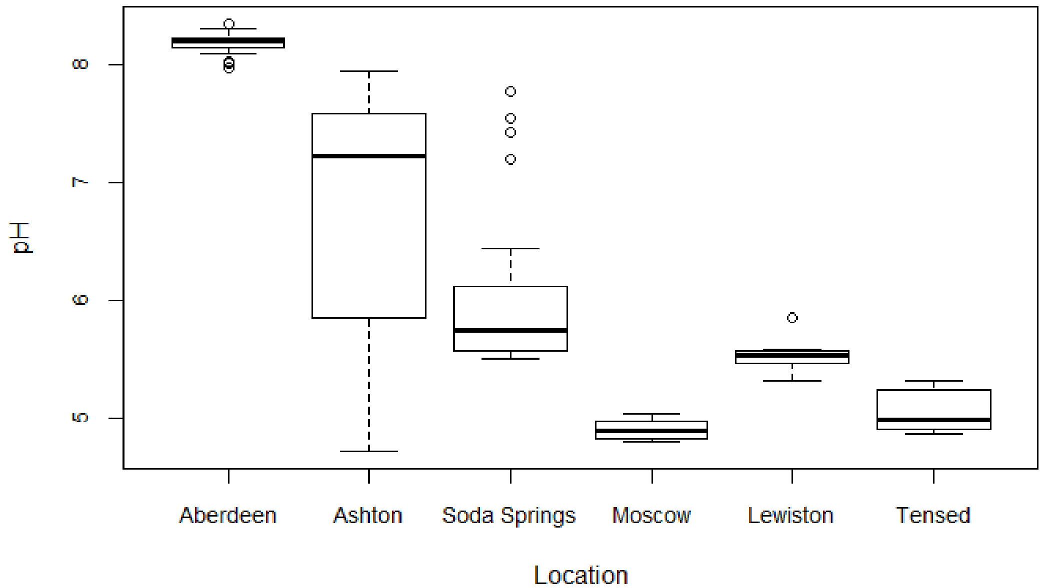
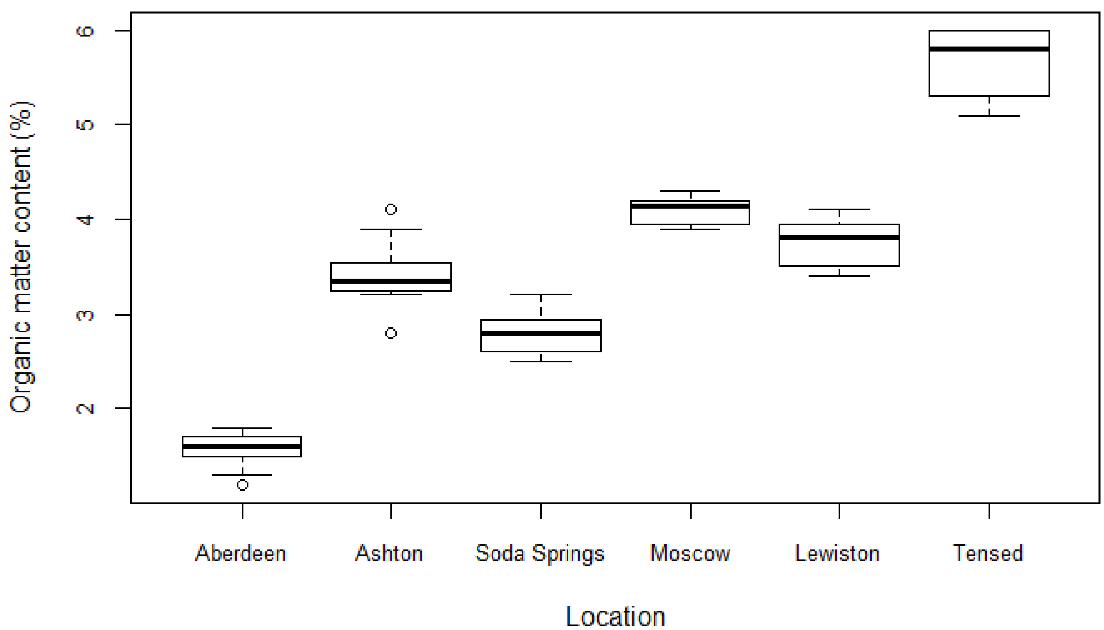
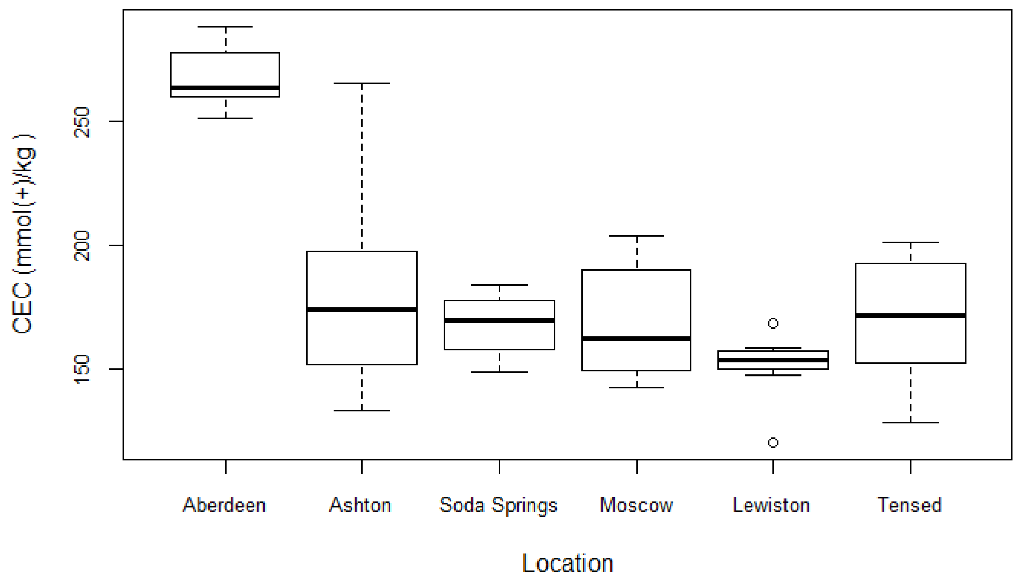


Figure 2.4 The variety comparison of marginal estimated means for northern Idaho samples. Error bars are the standard error

The different soil properties at each site were evaluated (Appendix Table 2.14) and the means were significantly different in soil variables by region except Olsen P, NO_3-N, and NH_4^+-N (Appendix Table 2.17). The pairwise comparison of location for each variety were evaluated using Tukey HSD (Appendix Table 2.18). The highest average soil pH occurred in Aberdeen soils and lowest pH occurred in the Moscow soils. Tensed soils had the highest amount of organic matter, Aberdeen had the highest CEC in soils (Figure 2.5). The mean concentrations of Cl^- and NO_3^--N in soils in the southern Idaho region soils are significantly higher than in northern Idaho soils. Soil P and NH_4^+-N concentrations in both regions are not significantly different.


pH
8
7
6
5
Aberdeen
Ashton
Soda Springs
Moscow
Lewiston
Tensed
Location


Organic matter content (%)
6
5
4
3
2
Aberdeen
Ashton
Soda Springs
Moscow
Lewiston
Tensed
Location



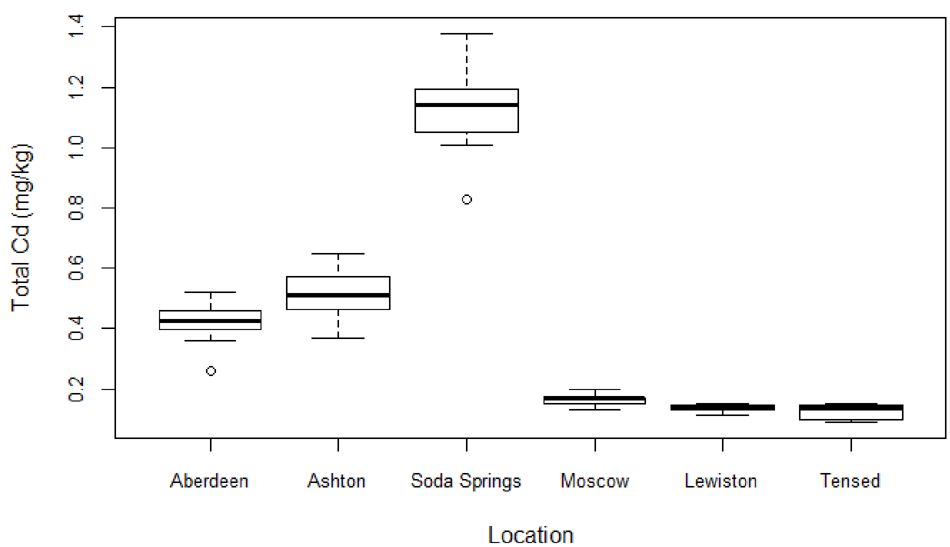


Figure 2.5 pH, organic matter content, and CEC, total Cd variations at different sampling locations; Aberdeen, Ashton, and Soda Springs are located in the southern Idaho region and Tammany, Moscow, and Tensed locations are in northern Idaho.

Bioaccumulation Factors

The bioaccumulation factors (BAF) were calculated by dividing grain Cd by soil Cd for paired soil-grain samples. Appendix Table 2.15 summarizes the mean BAF and Figure 2.6 presents the variation of BAF in each variety. BAF varies between 0.13-0.16 for the different varieties, except Brundage. UI Brundage had the highest BAF and is significantly greater than the other varieties (Table 2.6); approximately three-times greater than the BAF of other varieties. Grain Cd concentrations in Brundage are significantly different at the three northern Idaho locations (Figure 2.4).

Table 2.6 Pairwise comparison of bioaccumulation factor and variety

	Mean difference	95% confidence level lower boundary	95% confidence level upper boundary	p adj
CdDH-026-CdDH-016	0.00957	-0.0881	0.107	0.999
CdDH-028-CdDH-016	-0.00124	-0.0989	0.0964	1.000
CdDH-266-CdDH-016	0.00205	-0.0956	0.0997	1.000
LCS Star-CdDH-016	0.0209	-0.0767	0.119	0.998
UI Brundage-CdDH-016	0.2614	0.164	0.359	0.000*
UI Platinum-CdDH-016	0.0118	-0.0858	0.110	0.999
UI Sparrow-CdDH-016	0.0255	-0.0721	0.123	0.992
CdDH-028-CdDH-026	-0.0108	-0.108	0.0869	0.999
CdDH-266-CdDH-026	-0.00752	-0.105	0.0901	0.999
LCS Star-CdDH-026	0.0114	-0.0863	0.109	0.999
UI Brundage-CdDH-026	0.252	0.154	0.349	0.000*
UI Platinum-CdDH-026	0.00228	-0.0954	0.0999	1.000
UI Sparrow-CdDH-026	0.0159	-0.0817	0.114	0.999

CdDH-266-CdDH-028	0.00329	-0.0944	0.101	1.000
LCS Star-CdDH-028	0.0222	-0.0758	0.120	0.997
UI Brundage-CdDH-028	0.263	0.165	0.360	0.000*
UI Platinum-CdDH-028	0.0131	-0.0845	0.11075	0.9998930
UI Sparrow-CdDH-028	0.0268	-0.0709	0.124	0.989
LCS Star-CdDH-266	0.0189	-0.0788	0.117	0.999
UI Brundage-CdDH-266	0.259	0.162	0.357	0.000*
UI Platinum-CdDH-266	0.00980	-0.0880	0.107	0.999
UI Sparrow-CdDH-266	0.0235	-0.0741	0.121	0.995
UI Brundage-LCS Star	0.240	0.143	0.338	0.000*
UI Platinum-LCS Star	-0.00909	-0.107	0.0885	0.999
UI Sparrow-LCS Star	0.00459	-0.0931	0.102	0.999
UI Platinum-UI Brundage	-0.250	-0.347	-0.152	0.000*
UI Sparrow-UI Brundage	-0.236	-0.334	-0.138	0.000*
UI Sparrow-UI Platinum	0.0137	-0.0840	0.111	0.999

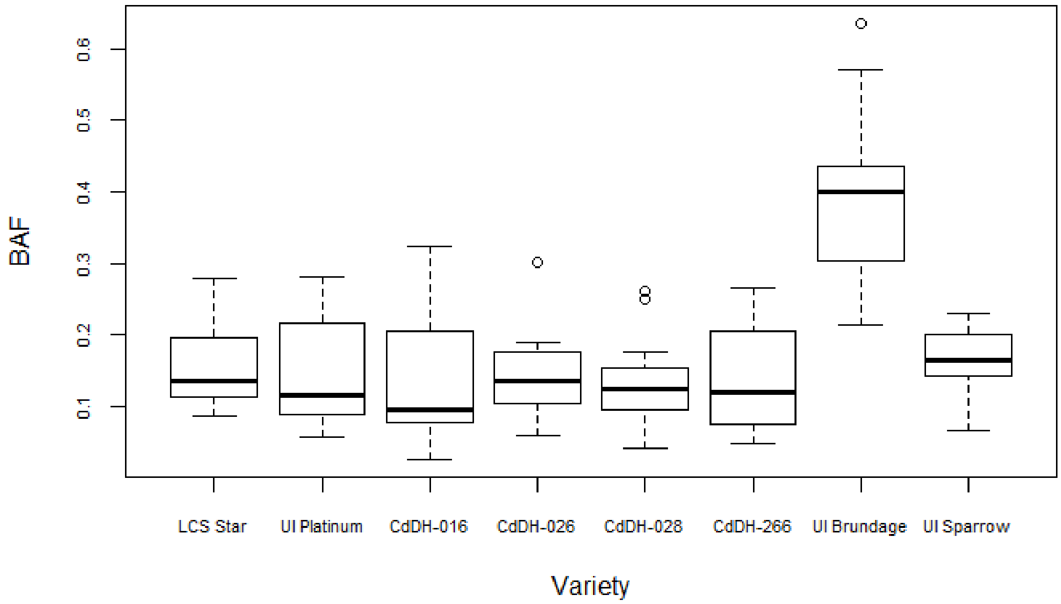


Figure 2.6 Bioaccumulation factor (BAF) in each wheat variety.

Principal Component Analysis

Principal component analysis was conducted to determine the relationships between soil variables. This analysis is helpful to reduce the dimensionality of large data sets by transforming variables into a smaller set of principal components (PC) and relating the independent variables to the PC. The first PC is the combination associated with the greatest variance in the dataset, and each subsequent PC is associated with progressively less variance. Subsequent PCs must be orthogonal to the preceding PCs. Grouping the variables in this way reduces the number of dimensions and guarantees they will not be collinear (Powell and Lehe, 2015).

In PCA, each variable is assigned a coefficient, or a loading, that reflects its contribution to the PCs. Using the loadings, the coefficients of the linear combination of the initial variables from which the PC are constructed can be interpreted, and the sign of a loading indicates whether a variable and a PC are positively or negatively correlated (Holland, 2019).

PCA groups variables into linear combinations, or components. The coefficient values shown in Table 2.7 and loadings equal to -1 or 1 indicate that the factor strongly influences the variables. Figure 2.7 graphically represents the correlation between soil variables with PC1 and PC2. Variables are clustered according to their loadings. Sand percentage, pH, and CEC, EC, sulfate, and Cl are negatively correlated with both PC1 and PC2, and other soil variables are positively correlated with PC1. pH, DTPA Cu, DTPA Mn, DTPA Fe, total Cu, total Fe, total Mn, total N, organic carbon, organic matter content, CEC, percentages of sand, silt, and clay are the major contributors to PC1. The major contributors for the PC2 were DTPA Cd, DTPA Zn, total Cd, total Zn, total P, NH_4^+-N, NO_3^--N, and SO_4^{2-}-S.

Table 2.7 Correlations between variables and components in soils. Higher correlations imply a variable contributes more to a PC. The proportion of variance refers to the percent of overall variance that each PC describes.

	PC1	PC2	PC3	PC4
pH	-0.267	-0.0918	0.0404	-0.0245
EC	-0.0859	-0.0952	0.461	0.124
DTPA Cd	0.149	-0.389	-0.0968	0.0632
DTPA Cu	0.205	-0.148	-0.232	-0.167
DTPA Fe	0.251	0.131	0.0312	-0.208
DTPA Mn	0.243	-0.0747	-0.115	0.0948
DTPA Zn	0.0115	-0.224	0.0761	-0.190
Total Cd	0.0635	-0.443	-0.0990	0.0776
Total Zn	0.153	-0.326	0.158	0.00320
Total Cu	0.209	-0.0733	0.00570	0.153
Total Mn	0.279	-0.0522	0.0611	0.0956
Total P	0.0829	-0.332	-0.0151	-0.400
Total Fe	0.232	0.246	0.141	0.104
Organic matter	0.233	0.232	0.146	-0.163
Olsen P	0.113	-0.132	0.337	-0.1692
CEC	-0.262	-0.0264	-0.00940	-0.148
Organic carbon	0.216	0.235	0.174	-0.226
Total N	0.231	0.137	0.242	-0.225
NO3_N	0.0415	-0.138	0.463	0.207
NH4_N	0.148	-0.250	-0.0748	-0.103
Cl	-0.146	-0.0793	0.223	0.130
K	0.128	-0.00890	-0.0141	0.618
SO42_S	-0.156	-0.140	0.375	-0.0575
% sand	-0.274	-0.00990	0.00960	-0.0821
% silt	0.267	0.0215	0.0246	0.0777
% clay	0.239	-0.0223	-0.0960	0.0771
Standard Deviation	3.338	2.10	1.71	1.23
Proportion of variance	0.428	0.169	0.111	0.0586

Cumulative Proportion	0.428	0.598	0.709	0.767

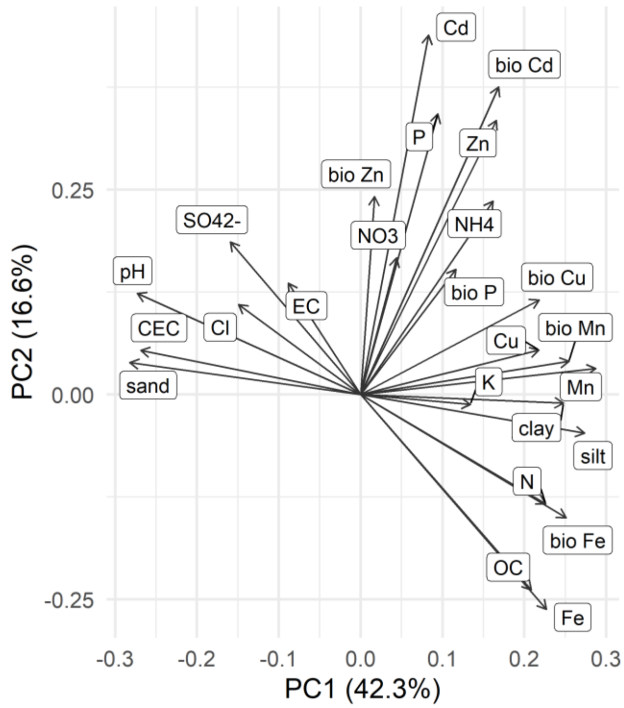


Figure 2.7 Correlations between variables and PCs. Longer lines indicate greater variation. The lines which are closer to each other, the more correlated they are. “bio” refers to the bioavailable metals extracted by DTPA analysis. Element symbols are total soil metal concentrations.

Stepwise Regression Analysis

Among the 25 soil properties tested in the stepwise regression, total soil Cd, DTPA Cd, total P, Olsen P, organic C, total N, and NO_3^--N were selected as the most significant soil factors affecting Cd concentration in wheat grain (Table 2.8). Grain Cd decreased with Total Cd, Total P, and organic C, and increased with others selected soil properties. Region (southern and northern Idaho) also significantly affect grain Cd concentration. The variable importance is calculated from producing the largest t-statistics for each variable and it indicates the relative significance of the soil properties as predictors for Cd concentration of wheat grain. DTPA Cd is the most significant factor for predicting grain Cd concentration, followed by Olsen P and total Cd. Since this model has a high coefficient of regression (R^2 = 0.81) and a very low p-value, it is accurate for predicting wheat grain Cd concentration in Idaho. Figure 2.8 shows the linear relationship between predicted values from the model and the grain Cd. The dotted lines in this graph are the baby food cut-off level of Cd in grain, which is 0.03 mg/kg. When including the "variety" effect for this model, the model is improved when Brundage and LCS Star (higher Cd loading varieties) are included in the model, resulting in an R^2 = 0.87. The coefficient values for the model that includes variety are listed in Table 2.9.

Table 2.8 Summary output of the stepwise regression to predict significant soil properties (among 27 variables) that affect the grain Cd concentration.

	Variable importance	Estimate	Std. Error	t value	Pr(>|t|)
Intercept		6.36e-02	1.26e-02	5.04	2.56e-06
DTPA Cd	7.18	3.05e-01	4.24e-02	7.18	2.19e-10
Olsen P	5.40	6.22e-04	1.151e-04	5.41	5.60e-07
Total Cd	4.53	-9.65e-02	2.13e-02	-4.53	1.89e-05
Region	2.96	2.83e-02	9.55e-03	2.96	0.00395
Organic C	2.69	-5.72e-02	2.13e-02	-2.69	0.00857
Total P	2.28	-3.90e-01	1.71e-01	-2.28	0.0252
NO_3^--N	2.17	2.05e-04	9.44e-05	2.17	0.0329
Total N	2.08	5.68e-05	2.73e-05	2.08	0.0402

Variable importance is the relative significance of soil variables to predict the grain Cd concentration.

Residual standard error: 0.01727 on 87 degrees of freedom
Multiple R-squared: 0.8261, Adjusted R-squared: **0.8101**
F-statistic: 51.65 on 8 and 87 DF, p-value: < 2.2e-16

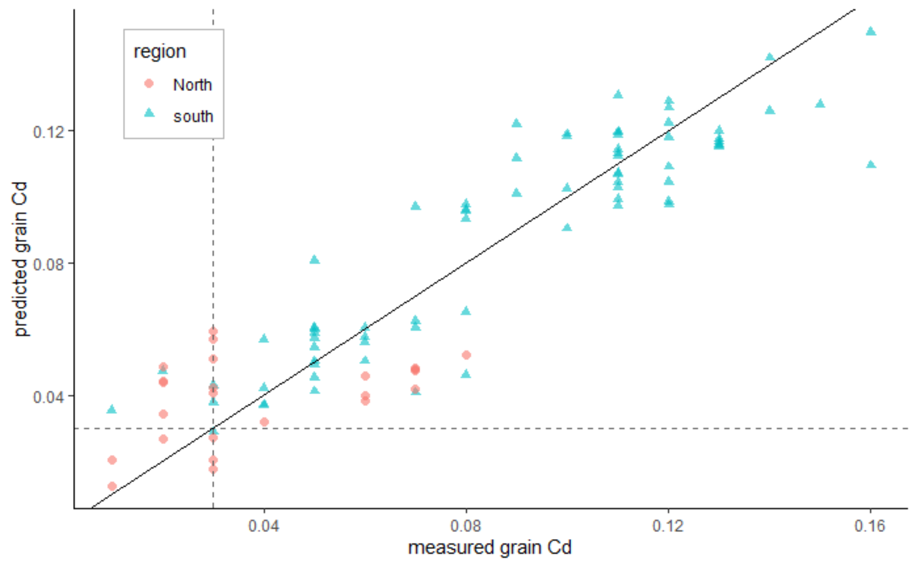


Figure 2.8 Grain Cd vs predicted values from the stepwise regression model (R^2=0.81).

Table 2.9 Summary output for the stepwise regression model with variety included.

	Estimate	**Std. Error**	**t value**	**Pr(>|t|)**
(Intercept)	4.60e-02	1.12e-02	4.09	0.000101
DTPA Cd	3.17e-01	3.62e-02	8.76	2.42e-13
Olsen P	6.77e-04	9.92e-05	6.82	1.48e-09
Total Cd	-1.04e-01	1.82e-02	-5.73	1.67e-07
Total P	-3.19e-01	1.46e-01	-2.18	0.0319
Organic C	-5.58e-02	1.83e-02	-3.06	0.00302
Total N	5.03e-05	2.33e-05	2.16	0.0341
NO_3^--N	1.80e-04	8.35e-05	2.16	0.0336
Region Southern	3.79e-02	9.48e-03	3.99	0.000142
Variety LCS Star	1.24e-02	5.97e-03	2.08	0.0410
Variety UI Brundage	3.58e-02	6.00e-03	5.96	6.31e-08

Residual standard error: 0.01456 on 81 degrees of freedom
Multiple R-squared: 0.8849, Adjusted R-squared: **0.865**
F-statistic: 44.48 on 14 and 81 DF, p-value: < 2.2e-16

Random Forest Analysis Model

Random Forest analysis evaluates the relative importance of each variable on the grain Cd prediction by looking at how much the tree nodes that use that variable reduce impurity across all trees. Since this analysis is done on a subset of the dataset, it prevents data over fitting. This analysis can be used for both regression and classification tasks, and it is easy to view the relative impacts of soil variables on grain Cd concentration.

In order to determine the parameter importance, MSE and Node Purity were evaluated. Appendix Table 2.20 lists Inc MSE (Incremental Mean Squared Error) for the variables; MSE increases if the variable is completely randomized, which provides a relative indicator of the importance of the variable for predicting grain Cd concentration. Node purity is a quantitative measure of the variable homogeneity in a node. DTPA Cd, total Zn, Olsen P, DTPA Fe, and total Cd have greater impact on node purity compared to the other variables, while total P, clay content, EC are less important for predicting grain Cd content (Figure 2.9). For this model R^2 value is 0.78, suggesting the Random Forest analysis is a good model to fit the data. Figure 2.10 shows the linear relationship between predicted values from the model and the measured grain Cd concentration. The dotted lines in this graph are the industry cut-off level of Cd in grain, which is 0.03 mg/kg and the solid line indicates where a perfect correlation between points would occur.

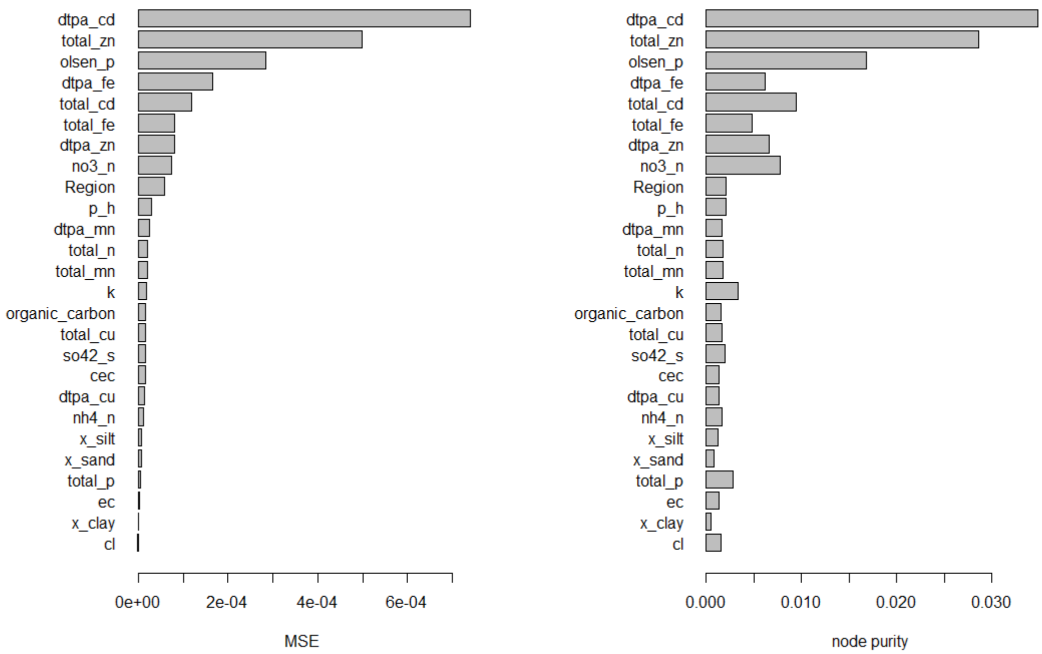


Figure 2.9 Mean squared error and node purity variation of each variable.

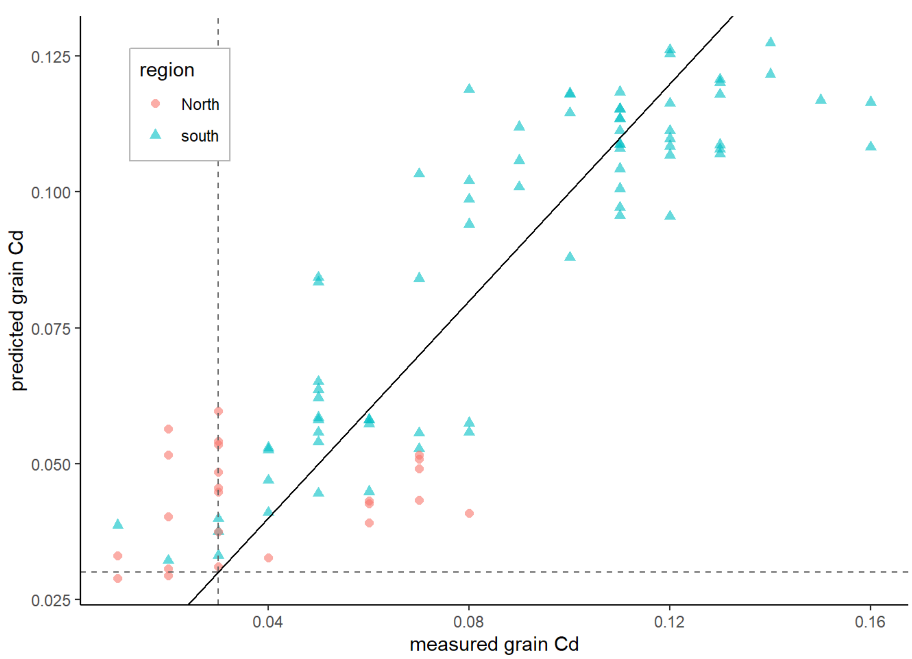


Figure 2.10 Predicted grain Cd vs measured grain Cd (in mg/kg) in Random Forest Method (R^2 = 0.78).

Partial Least Square (PLS) Regression

Multiple-linear regression can be used with many variables to build a model for a specific purpose. PLS modeling avoids over-fitting issue from over-parameterization that makes multiple linear regression modeling indiscriminate. The PLS model extracts latent factors that account for most of the variation in the response variable (Tobias, 2016). PLS modeling is used to find the fundamental relations between two spaces of X and Y. The PCA is followed by a regression step, where the decomposition of X is used to predict Y (Abdi, 2007).

The correlation output from the PLS regression on the soil and grain data is shown in Figure 2.11. As the Y variable grain Cd concentration was chosen, and soil property variables were changed from -1 to 1, where larger vectors in the same domain as grain Cd are more significant predictors. Figure 2.11 shows that total Cd, DTPA Zn, NO_3^-N, total P, bioavailable P, total Zn, and DTPA Cd are important predictors for Cd in grain. For this model R^2 value is 0.84, which is the amount of variance in grain Cd concentration explained by independent variables. Figure 2.12 shows the variable importance, which is an indicator of the significance of the variables for predicting grain Cd concentration and Table 2.10 presents the regression coefficient of each variable in the model. Figure 2.13 shows the linear relationship between actual grain Cd and predicted grain Cd from the PLS model.

Table 2.10 Regression coefficient of each variable for predicting grain Cd form PLS modeling.

Variable	Regression Coefficient	Variable	Regression Coefficient
pH	-1.65e-02	Olsen P	1.77
EC	-1.98e-01	CEC	-4.69e-01
DTPA Cd	2.46	Organic C	7.98e-02
DTPA Cu	1.09	Total N	-1.33
DTPA Fe	-5.29e-01	NO_3^--N	-1.15e-03
DTPA Mn	-5.71e-02	NH_4^+-N	2.59e-01
DTPA Zn	-3.15e-02	Cl	3.47e-03
Total Cd	-1.38	K	2.59e-01
Total Zn	9.68e-01	SO_4^{2-}-S	5.50e-02
Total Cu	8.13e-02	% Sand	5.28e+14
Total Mn	5.61e-01	% Silt	4.03e+14
Total P	-6.47e-01	% Clay	1.56e+14
Total Fe	1.43	Region	8.677461e-01

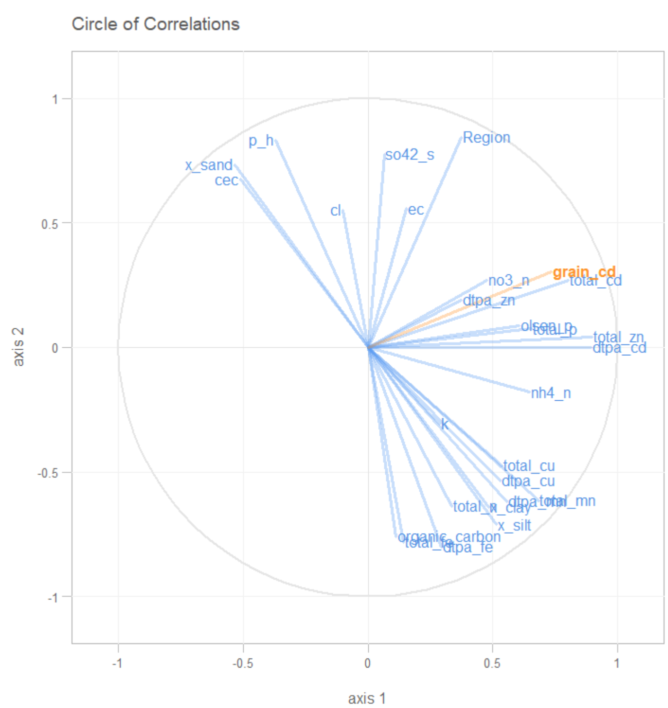


Figure 2.11 Correlations of each variable with grain Cd between fist two components.

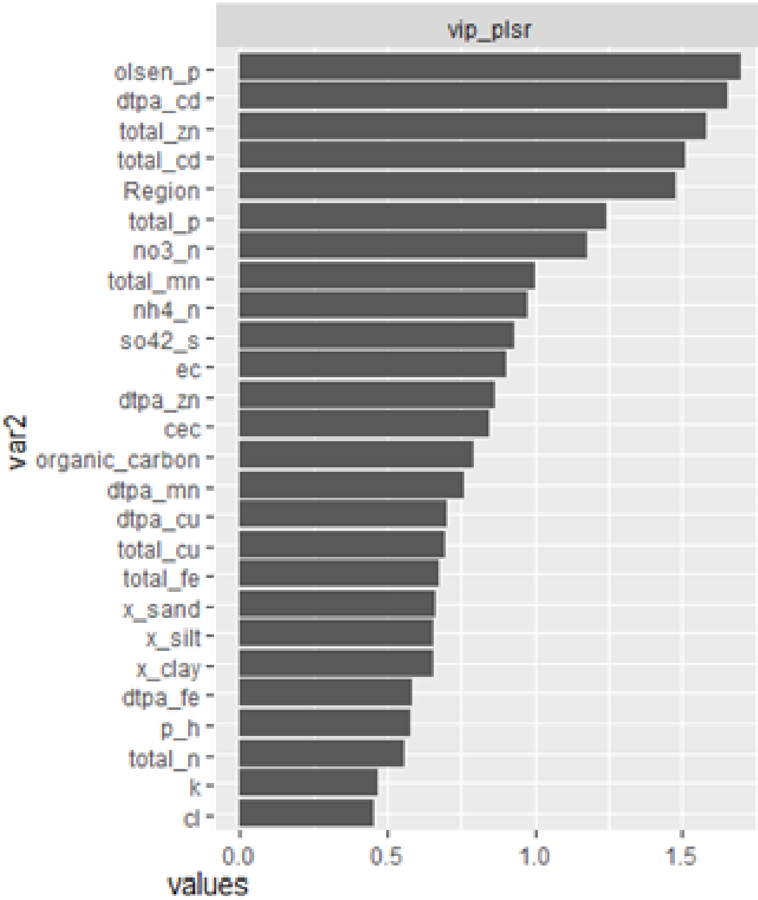


Figure 2.12 Variable importance of soil properties from Partial Least Square regression.

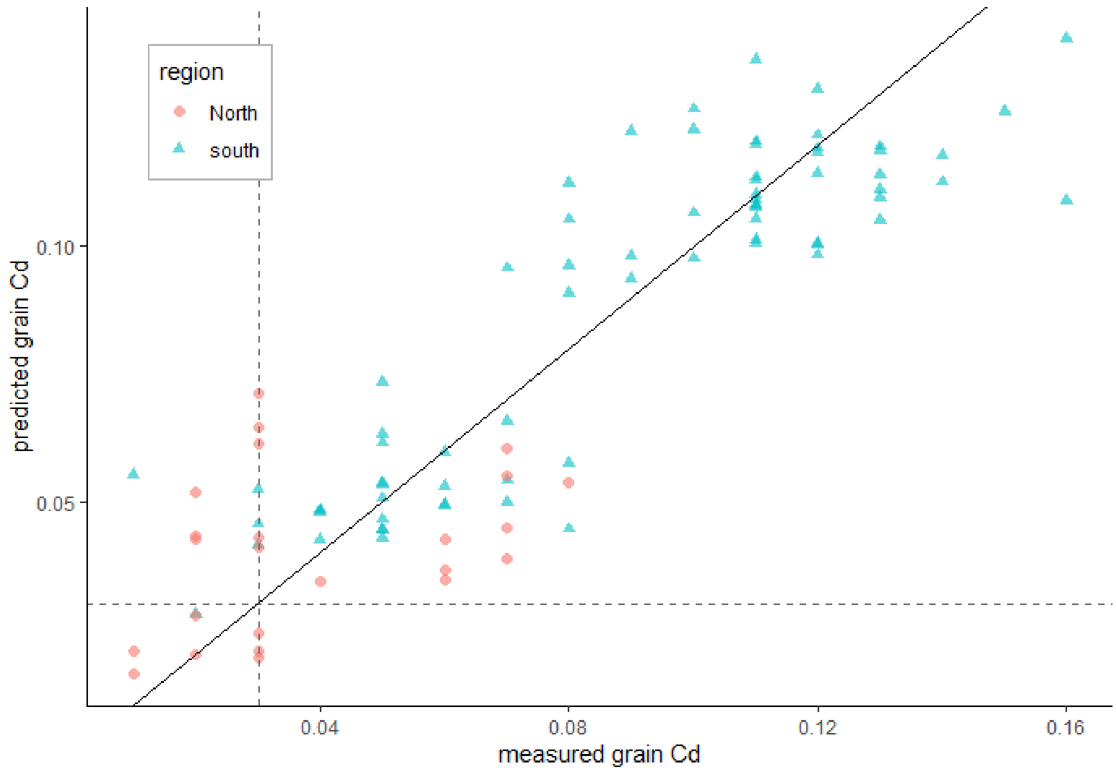


Figure 2.13 Predicted grain Cd vs measured grain Cd (in mg/kg) in PLS model (R^2= 0.84)

Table 2.11 Variable importance ranking of Stepwise Regression model, Random Forest model, and PLS model.

Rank	**Step Reg model**	**Random Forest model**	**PLS model**
1	DTPA Cd	DTPA Cd	Olsen P
2	Olsen P	Total Zn	DTPA Cd
3	Total Cd	Olsen P	Total Zn
4	Region	DTPA Fe	Total Cd
5	Organic C	Total Cd	Region
6	Total P	DTPA Zn	Total P
7	NO_3^--N	NO_3^--N	NO_3^--N

Discussion

Grain is the source of 26.9% of the dietary exposure to Cd, followed by vegetables (16.0%), and starchy roots and tubers (13.2%) (ESFA, 2012). Among wheat classes, Durum wheat uptakes more Cd than other wheat classes (Wangstrand et al., 2006). The Cd concentration in the grain samples grown in Idaho varies by wheat varieties. Grain Cd concentration of Brundage and UI Sparrow (both grown in northern Idaho) is less than the other varieties (Figure 2.2). Among these two, UI Sparrow had the lowest Cd concentration. LCS Star grain in Soda Springs had the highest average Cd concentration followed by LCS star in Ashton and CdDH-026 planted in Ashton and Soda Springs. Greger and Lofstedt (2004) studied Cd uptake by different wheat varieties and the results showed that there are differences among the cultivars in the ability to accumulate Cd in grains. It may be due to the variation of accumulation of Cd in roots and translocation from roots to shoots and variation of the Cd concentration in the shoots, flag leaves, and seed coats.

Figure 2.3 shows that Cd concentrations by the same varieties differ between locations, which is due to the differences in soil properties. In southern Idaho, wheat grain from Soda Springs and Ashton had the same average Cd concentration, which were greater than Aberdeen. In northern Idaho, Tensed grain had the lowest average Cd concentration. The different concentration in wheat is due to the variation of soil and site properties. PCA analysis showed that several components were required to account for the variability, but the two principal components that accounted for the most variance had DTPA Cd and Zn, total Zn, Cd and P, and available nitrate and ammonia in one domain, and the other 18 soil variables in a second domain. The alignment of the former listed domain suggests some commonality among these factors between soils, which happen to also be the best predictors for all three of the fitting models. To determine the measurable soil properties most important for Cd uptake in the Idaho wheat, three different models were used to predict grain Cd concentration. The stepwise regression model uses t-statistics to determine the variable importance and the model results indicate that DTPA Cd> Olsen P> total Cd> region> organic C> total P> NO_3^--N >total N are important factors that affect grain Cd concentration. Among significant soil properties, DTPA Cd and Olsen P are the most significant factors. According to the Random Forest model (Appendix Table 2.20 and Figure 2.19) DTPA Cd is the most important variable for predicting grain Cd concentration, followed by total Zn, Olsen P, DTPA Fe, and total Cd. Total P, clay content, EC have less impact on grain Cd concentrations. And PCA also indicated that, DTPA Cd is positively correlated with total Cd, total P, Olsen P, and total Zn (Figure 2.7). The Random Forest model results are different from the stepwise regression model. The PLS regression model predicts Olsen P> DTPA Cd> total Zn> total Cd> region>total P>NO_3^--N as the order of variable importance for predicting grain Cd concentration. Among soil variables, all three of the models selected DTPA Cd, and Olsen P as the most important predictors for Cd concentration (Table 2.11).

Both Stepwise regression model and Random Forest model were applied to a dataset that included the base data set and two new sites and new varieties grown in Idaho. Results from the two models agreed with the results from the base data set, illustrating the flexibility of the model for predicting grain Cd uptake based on soil properties (Appendix Figure 2.23, 2.24).

DTPA Cd and Olsen P extractions are designed to measure bioavailable Cd and P, respectively, and are significant factors in predicting grain Cd concentration. Tracy and Sheila (2006) also reported that extractable Cd content in soil may be an improved indicator of bioavailability and toxicity than the total Cd concentration. The availability of Cd and Zn differed among soil types (Appendix Table 2.14). Total Cd in the soil is an important measurement, but for plant uptake, the bioavailability fraction in the soil is more important (Ding et al., 2013). Total soil Cd content is not necessarily a good predictor of bioavailability because Cd binding with the soil particles, and thus availability, varies with the soil properties. For example, loamy soils have a greater capacity to adsorb metals than sandy soils (Scokart et al., 1983), and geochemical and biogeochemical processes such as podsolization alter metal species and distribution within the soil profile (Rieuwerts et al., 1998).

Olsen P and total P are good predictors to determine Cd concentration of wheat grain from all three models. Bray-P1 and Olsen methods are the most widely used soil test P methods. Bray-P1 test is reliable on neutral or acid soils but that it tends to underestimate available P on calcareous soils. The Olsen test is more reliable for calcareous soils. Bray test would be more efficient test for acidic soils, but Olsen has also been shown in some research to be reasonably effective extracting P from acidic soils (Olsen, et al., 1954; Ara et al., 2018). In this study the Olsen was used for all soils as an estimate of available P. The correlation between total P and Cd could indicate Cd contamination in P fertilizer. The effects of P fertilization on Cd concentration may be influenced by the crop species, the Cd concentration in the fertilizers, and the interactions among P, Zn, and Cd during uptake and translocation within the crop (Grant et al., 1999). Correlations of total Cd concentrations in the soils with nitrate, ammonium, and sulfate concentrations may also be due to covariates because soils that have high P fertilizer application also likely have high concentrations of other fertilizers. Application of P as reagent grade phosphate increased Cd concentration and Cd accumulation in both flax seed and durum wheat grain (Jiao et al., 2004), suggesting that fertilizer applications enhance Cd uptake in wheat.

Total N, nitrate, and organic C in the soil were selected by all models as significant variables for wheat grain Cd concentration prediction. Nitrogen fertilization has been shown in other studies to improve Cd uptake. Cheng et al. (2017) reported that ammonium-based N favors Cd phytoextraction in the *Carpobrotus rossii* plant. Hattab et al. (2014) found that the N supply may improve the uptake rate of Cd by alfalfa. Ata-UI-Karim et al. (2019) showed a positive correlation of plant phenology and yield with grain Cd concentration and soil properties under varied N application rates. Erikkson (1990) reported that ammonium fertilizers reduce the soil pH, which may result in increased availability of Cd for uptake by plants. Cadmium concentration was also shown to increase in spinach, oats, and radish upon N fertilizer addition in high pH soils (Kashem and Singh, 2002).

A recent study revealed that the application of organic N decreased Cd accumulation in cucumber and increased the biomass of Cd stressed plants compared to plants with the application of inorganic N (Dresler et al., 2021). Organic carbon also has an indirect effect on phytoavailable soil Zn and Cd concentrations and the uptake of these metals by wheat in addition to direct inputs with fertilizers and other amendments (Roman et al., 2017). So, fertilizers play a major role in the Cd accumulation of wheat grain.

Soil properties pH, Cl, CEC, other soluble metals, and elements have been shown to be important to predict the Cd concentration in plants. However, they were not correlated with plant Cd concentration in this study. Soil pH was not a significant factor to predict grain Cd uptake for the full dataset (both northern and southern data) in our study and also it was not an important variable to predict grain Cd in southern Idaho (Appendix Table 2.21). Soil pH has been observed to be an important factor in plant Cd uptake by many researchers (Speir et al., 2003; Oliver et al., 1994; Page et al., 1987). In contrast, Wenzel et al. (1996) showed 80% of the Cd accumulation in wheat grain from soil with typical Cd levels were explained by cultivar, total soil Cd, and organic carbon (pH was not a significant predictor for full dataset). Baize et al. (2009) also found that soil pH was not needed to model Cd concentrations in wheat grain from soils that had natural Cd input (i.e., uncontaminated). The soil pH ranges are between 4.5-8.5, and thus a high range of pH values was included in the model prediction in full dataset. Among the sampling sites, Aberdeen soil has the highest pH (strongly alkaline) (Figure 2.5). Northern Idaho soils have lower soil pH than most southern Idaho soils. Soils in Moscow are more acidic than soils in Tammany (4.9 vs 5.4, Appendix Table 2.14).

The uptake of Cd is mainly regulated by the free Cd^{2+} activity in soil solution. At low pH, dissolved Cd is higher, and Cd uptake by plants has been observed to be related to soil pH (Speir et al., 200, Page et al., 1981, Oliver et al., 1998). Eriksson (1989) observed that Cd content of rapeseed plants grown in Cd-polluted soil was markedly higher at pH 4.0 than at pH 5.0 because in acidic soils Cd exists as free Cd^{2+} ions. Ross (1994) observed that in alkaline soil pH conditions (pH>7), Cd uptake in wheat grain decreased due to (co)precipitation as carbonates and other minerals. Despite the importance of pH on metal speciation in soils, in this study, pH was not selected as a significant predictor for grain Cd concentration by any of the multivariate models, even though soil pH had a range of nearly four units (4.5-8.5).

Cation exchange capacity (CEC) is a significant soil property that affects metal mobility and bioavailability in soil. CEC depends on the density of negative charges on the surfaces of soil colloids and the relative charges of metal species in solution and on the soil surface (Evans, 1989). The CEC of soil depends on organic matter content, clay type, and clay content. Generally, the higher the CEC, the greater the ability to retain heavy metals. In a study in Iran, a positive correlation ($p < 0.05$, R^2=0.593) was observed between Cd concentration in wheat and soil CEC (Jafarnejadi et al., 2011). However, CEC was not selected as an important predictor for Cd concentrations in wheat grain in the current study.

Chloride concentration in soil is an important consideration for Cd uptake by plants. Chloride can be added to the amendment soil by phosphate fertilizers, urine, and biogas digestates (Dahlin et al., 2016). Smolders et al. (1998) used a pot experiment to test the effects of chloride on Cd uptake in Swiss chard by adding NaCl into Alfisols. With the increase of NaCl concentration, the Cd concentration increased from 65 to 400 nmol L^{-1} in soil solution and then increased the Cd uptake by the plant. Hattori et al. (2006) found that sunflower and Kenaf leaves uptake twice as much Cd with the application of Cl^- compared to the controls. In contrast, Smolders et al. (1998), Hattori (2006), Norvell et al. (2000) studied Cd uptake in Durum wheat in North Dakota and observed that the Cd content in grain was greatest at a low level of Cl^-. Chloride ions make complexes with Cd in soil solution, such as $CdCl^+$, $CdCl_3^-$, $CdCl_4^{2-}$ causing total Cd concentration to increase in soil solution (Traina, 1999). Weggler et al. (2004) found that the chemical species $CdCl^+$ in soil has a positive correlation with the Cd uptake of plant shoots in bio-solid amended soils. But Cl^- availability is not a significant predict the Cd concentrations in wheat in this study.

Total soil Zn and DTPA Zn were not significant factors to predict Cd concentration in wheat in the Stepwise regression model. But both the Random Forest model and PLS model selected total and DTPA Zn as significant for prediction of grain Cd concentration. Many studies have shown the relationship between Cd and Zn for Cd uptake by plants (Rizwan et al., 2017; Oliver et al., 1994; Sadana and Singh, 1987; Podar et al., 2004). Rizwan et al. (2019) and Zare et al. (2018) found that the increasing level of Cd is drastically reduced by the application of a low dose of Zn. Further, Podar et al. (2004) suggested that the human health risk from consuming plant parts grown on Cd-contaminated substrates is lower when Zn is also present in *Brassica juncea*. Interactions between Cd and Zn can happen during the plant uptake, transport within plants, and accumulation of Cd within edible tissues (Narwal et al., 1993; Pence et al., 2000). The PLS model placed total and DTPA extractable Zn vectors in the same quadrant as grain Cd, suggesting that the Zn in the soil does not inhibit Cd uptake by wheat grain.

Considering the most common variables among the three models, DTPA Cd, DTPA Zn, Olsen P, total Cd, total Zn, nitrate-N, and total P can be used as the best soil parameters for predicting Cd concentration in grain and to select sites for growing ultra-low Cd wheat. These soil measurements represent soil factors that significantly affect Cd uptake by wheat in Idaho.

Conclusion

Grain Cd concentration in wheat samples were different by location and variety. The wheat grown in northern Idaho had a lower Cd content compared to southern Idaho regions and UI Sparrow wheat variety grown in Tensed had the lowest uptake of Cd. The pairwise difference between UI Sparrow and Brundage wheat in northern Idaho sampling sites was significantly different and the variety enhanced the model accuracy to predict Cd uptake by wheat. These results conclude that, there is a large impact of variety on Cd uptake and that in some regions, it is dependent on soil properties. Learning more about their physiology could help researchers select genetic traits to reduce Cd uptake. These results point toward a need for more research regarding the influence of variety on the Cd uptake by wheat. Variety selection, and supplementation with nutrients specific to uptake dynamics exhibited by each variety, could become effective Cd management tools.

Using advanced machine learning methods, this research investigated 25 soil variables and investigated their relation to soil Cd. Utilizing these advanced data analytical methods allowed for prediction of which soil properties are the most important for predicting Cd grain concentration in wheat, and from this important soil processes and management can be determined. Previous research indicated soil pH, SOM and Cl^- were critical soil measurements for predicting Cd bioavailability from soils for plants. The current research does not support this conclusion. Likewise, relations to soil Zn have also been shown to be important but was only of minor importance in the modeling done in this study. Instead, multiple regression of the data using Stepwise regression model, Random Forest model, and PLS regression model revealed that DTPA Cd, Olsen P, total Cd, DTPA Zn, total Zn, total P, total N, NO_3—N, and organic carbon were significant soil measurements related to wheat grain Cd concentrations. Among them, DTPA Cd and Olsen P were identified as the most significant predictors for wheat grain Cd concentration in Idaho. DTPA Cd concentration is a measure of the available Cd in the soil, and this research validated that is one of the most important soil measurement parameters for predicting which sites to grow low Cd containing wheat; DTPA Cd is even more important than total soil Cd. The relation of grain Cd concentration to Olsen P may be indicative of P fertilizer application rates on soils, suggesting that careful management of phosphorous fertilizer application is required to achieve low-Cd concentrations in wheat grain. According to the study of Cd removal from the fertilizer, the solvent impregnated resin containing Cyanex-302 have been exhibited a good performance for Cd removal from 40% H_3PO_4 solution (Kabay et al., 2002).

Chapter 3: Evaluating accuracy of soil assessment methods at predicting Cd uptake in wheat

Introduction

Bioavailability is important to consider because it is becoming the most common method used for risk assessment. Bioavailability can be defined as the fraction of a chemical associated with soil and sediment to determine the exposure of plants and animals to the said chemical. A bioavailable chemical element presents or transforms into a free ion species which can then move through plant roots and affect plant growth and development (NRC, 2003).

Since heavy metal accumulation in plants is a serious problem, several studies were performed to find a suitable method to estimate bioavailability of selected metals. To determine the bioavailable Cd in soil, different extraction methods were used across different studies. The following table summarizes the results from various extraction methods in prior literature.

Table 3.1 Extraction methods and modeling methods.

Citation	Cd uptake by plant	Soil properties	Extract	Conclusion
Ding et al, 2013	Carrot	Stepwise multiple linear regression $\log[Cdcarrot] = 1.30 - 0.24pH + 1.27 \log[Cdsoil] - 0.40 \log[OC]$ $R^2 = 0.90$, $P < 0.001$	Stepwise multiple linear regression $\log[Cd_{carrot}] = 0.70 * \log[CaCl_2\ Cd] - 0.13$ $R^2 = 0.57$, $P < 0.001$ $\log[CaCl_2\text{-}\ Cd] = 1.66 - 0.31pH + 1.04 \log[Cd] - 0.70 \log[OC]$ $R^2 = 0.70$, $P < 0.001$	The multiple linear models for Cd content in carrot with total soil Cd, pH, and OC as predictors performed better than the model with $CaCl_2$ extractable Cd as a single predictor.
Wen et al, 2019	Rice	Stepwise multiple linear regression $\log[BCF] = -0.683 \times \log[\text{Soil Ca}] - 0.161 \times pH - 0.237$ ($R^2 = 0.478$, $P < 0.001$)	0.01M $CaCl_2$ is used to measure Cd uptake in rice ($R^2 = 0.237$, $P < 0.001$) DGT ($R^2 = 0.73$)	0.01M $CaCl_2$ extraction was not good. DGT-measured Cd provides a good estimation of Cd in rice grains.
Baize et al, 2009	Wheat		Canonical correlation and multiple linear regression analysis were used.	DTPA has less extraction capacity than EDTA, but a stronger than saline solutions, and is best adapted for neutral and alkaline pH values.

			CdW = – 1.21 – 0.011*CaCO3 – 0.173*MnS + 0.421*CdD – 0.145*ZnD + 0.214*CdN – 0.166*FSa + 0.111*CSa [CdD-DTPA extractable Cd; CDN- NH_4NO_3– extracted Cd; FSa – Fine Sand; CSa- Coarse sand] (R^2 = 0.502)	There was no comparison between extraction methods.
Wenzel et al, 1996	Wheat	Stepwise multiple linear regression $\ln(Y_{jk}) = \mu + \text{cultivar}_i + \beta{*}\text{Cd-t}_{jk} + \beta_2{*}\text{pHjk} + \beta_3{*}\text{OC}_{jk} + \beta_4{*}\text{Cl-soil}_{jk} + \beta_5{*}\text{ Ca-soil}_{jk} + \mathcal{E}_{jk}$ $R^2 = 0.904$, $P < 0.001$	CD-EDTA Cd-DTPA Cd-NH_4NO_3	Substituting Cd-t for Cd-EDTA or Cd-DTPA in type 2 models decreases R^2 by 10% to 30%. The low extractability of Cd by 1 M NH_4NO_3, is expected in these slightly acidic to alkaline soils.
Dia et al, 2017	*Brassica Chinensis*	Multiple linear regression method. Cd uptake by plant = f(pH, OC, Soil Cd)	Stepwise multiple linear regression DGT (R^2 = 0.95; p<0.01) Soil Solution (R^2 = 0.92; p<0.01) 0.05 mol·L^{-1} EDTA (R^2 = 0.89; p<0.01) 0.11 mol·L^{-1} HAc (R^2 = 0.84; p<0.01) 0.01 mol·L^{-1} $CaCl_2$ (R^2 = 0.82; p<0.01) Pseudo-total Cd (R^2 = 0.78; p<0.05)	DGT method > soil solution method > EDTA method > HAc method > $CaCl_2$ method > pseudo-total Cd method
Meers er al, 2007	Comparison of extraction methods		Horizon soil moisture samplers, 0.01M $CaCl_2$, 0.1M $Ca(NO_3)_2$, 0.1M $NaNO_3$, 1M NH_4NO_3, 1M NH_4OAc, 1M $MgCl_2$, 0.11M HOAc, 0.1M HCl, 0.5M HNO_3, 0.02M EDTA + 0.5M	$CaCl_2$ and $Ca(NO_3)_2$ methods work much more similarly in releasing Cd and they are better predictors than $NaNO_3$ method. NH_4OAc, HOAc, EDTA, HNO_3, HCl methods are significantly correlated with the aqua regia method and reflect the total soil content.

			NH_4OAc + 0.5M HOAc (pH 4.65), 0.005M DTPA + 0.01M $CaCl_2$+ 0.1M TEA (pH 7.3) and aqua regia	
Meers et al, 2006	Phaseolus vulgaris		Soil solution extraction by Rhizon soil moisture samplers, 0.01 M $CaCl_2$, 0.1 M $NaNO_3$, 1 M NH_4NO_3, 1 M NH_4NOAc, 1 M $MgCl_2$, 0.11 M HOAc, 0.5 M HNO_3, 0.1 M HCl, DTPA–TEA–$CaCl_2$, EDTA-NH_4OAc, and aqua regia.	Rhizon soil solution extractions, extractions based on unbuffered nitrate solutions and the diluted chloride solution $CaCl_2$ provided the best measure of Cd phytoavailability in the soil.
Ibaraki et al, 2005	Wheat		0.025M HCl, $MgCl_2$, DTPA, $Na_2P_4O_7$, 1M NH_4Cl	0.025M HCl extracted Cd significantly correlated with Cd in wheat and NH_4Cl extraction method also behave quite similar to HCl method. In comparison, other extraction methods are weakly correlated.

According to prior literature, the most common extraction method was 0.01M $CaCl_2$ to predict the Cd uptake by plants. But some results showed that it is not as good of a predictor of Cd availability when combined with soil properties. As an example, Ding et al. 2013 stated that the multiple linear models for Cd content in carrot with total soil Cd, pH, and OC as predictors performed better than the model with $CaCl_2$ extractable Cd as a single predictor. Similarly, Chaudri et al. 2007 also found that the stepwise addition of soil pH and OC to soil total Cd resulted in better prediction of wheat grain Cd concentrations (R^2 = 0.78), whereas their inclusion with NH_4NO_3 extractable Cd did not improve the relationship any further (R^2 = 0.56). However, the $CaCl_2$ method can release more Cd than 0.1M $NaNO_3$ as a single predictor. In comparison to divalent exchangeable cations, such as Ca, the monovalent cations NH_4, K and Na are less competitive for desorption of heavy metals from the soil matrix (Gommy et al., 1998). Since 1M $MgCl_2$ methods overestimated the exchangeable Cd because of chloride ions, researchers now use $Mg(NO_3)_2$ (Gommy et al., 1998).

Diffusive Gradients in Thin films (DGT) has been identified as an accurate predictor method because concentration gradient can be established at the soil-sampler interface using DGT, unlike in traditional soil extraction methods, which are mainly based on equilibrium concentrations related to the molarity of the extraction agent (Dai et al, 2017), which explains why there was no significant benefit in accounting for most of these soil factors separately in leaf Cd modeling. Gramlich et al. (2017) found that there was a significant relationship between DGT-available Cd and soil properties like pH, clay content, P, and available Fe for Cd uptake in cacao. Overall, DGT is an effective method that provides an in-situ means of quantitatively measuring labile species in the aqueous system. The principle behind that method is ensuring that transport of metal ions to an exchange resin is solely by free diffusion through a membrane of known thickness, Δg, and the concentration in the bulk solution, Cb, can be calculated from the measured mass in the resin, M, after time, t, by $Cb = M\Delta g/DAt$, where D is the molecular diffusion coefficient and A is the exposed surface area of the membrane (Zhang and Davison, 1995).

Generally, 0.005 M DTPA shows lower extractable levels than 0.02 M EDTA, which may be a result of the lower chelate concentration (Meers et al, 2007). EDTA can extract water-soluble Cd, exchangeable Cd, Fe, and Mn oxides combined with Cd, and organic matter–Cd complexes (Dai et al, 2017). Also, EDTA was originally developed for acidic soils so it shows a good correlation when investigating acidic soils (Kovacevic et al., 2002), but it seems to correlate poorly for neutral and alkaline soils. On the other hand, DTPA correlates poorly for acidic soils, but it can show a good correlation for neutral and alkaline soils (Feng et al., 2005).

When considering the acid-base extraction procedures, 0.1 M HCl, 0.5 M HNO_3, and aqua regia methods released Cd with similar strength, and results indicate that the pool extracted with these extractions is quite representative of the pseudo-total content (Meers et al, 2007). Ibaraki et al., (2005) also found that 0.025 M HCl extracted Cd was significantly correlated with Cd in wheat. The main objective of this chapter is to determine the best extraction methods to predict the Cd in wheat.

Methods and Materials

Soil Sampling

Soil samples were collected from Soda Springs, Aberdeen, Ashton, Rupert, and Kimberly from southern Idaho and Tensed, Moscow, and Tammany from northern Idaho. The top 15 cm of soil samples (n= 124) were air-dried and sieved through a 2-mm sieve.

DTPA Extraction Method

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 492 g for 10 minutes, and filtered through 0.22 μm polyethersulfone (PES) membrane filters. Extracts were analyzed on an ICP-OES for Cd, Cu, Fe, Mn, and Zn standardized using NIST traceable standards. The ICP MDL for the DTPA solution is estimated to be 0.004 mg/L Cd (using 3 times sigma).

Method Analysis for DGT

The DGT technique has been recently developed and used to measure labile species. Using DGT a concentration gradient can be established at the soil-sampler interface, which has advantages over traditional soil extraction methods that are based on equilibrium concentrations related to the molarity of the extraction agent. The DGT technique includes contributions from the liquid and solid phases of soil as well as the exchange dynamics between the two phases. The dynamic exchange of Cd from the solid to the liquid phase is an important factor that influences Cd uptake, and the Cd concentrations measured with DGT reflect these processes (Luo et al, 2010). The principle behind that method is ensuring that transport of metal ions to an exchange resin is solely by free diffusion through a membrane of known thickness, Δg. The concentration in the bulk solution, Cb, can be calculated from the measured mass in the resin, M, after time, t, by $Cb = M\Delta g/DAt$, where D is the molecular diffusion coefficient and A is the exposed surface area of the membrane (Zhang and Davison, 1995).

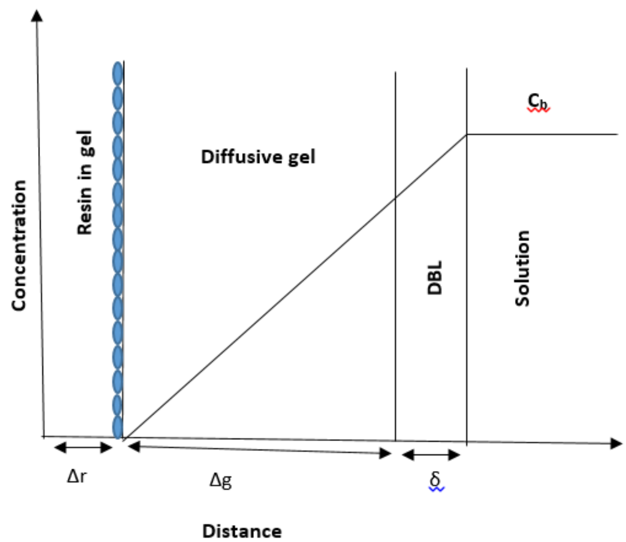


Figure 3.1 Schematic representation of the free concentration of ionic species in a hydrogel assembly in contact with the aqueous solution, where the concentration is Cb (DBL is diffusive boundary layer). The rate of diffusion is assumed to be the same in the gel and solution. (Zhang and Davison, 1995)

Extraction with the DGT method as described by Zhang and Davison (1995) was used as a measure of available metals. For this purpose, the below procedure was followed.

(1) Determining the water holding capacity of soil: For this, the percolation method was used. Briefly, 5, 10, 15, 20, and 25 g of soils were placed in a filter funnel and 25 ml of water was poured slowly over the soil in the funnel. As the soils got wet, the water trickled down to the cylinder. By measuring the collected water, the water holding capacity of each soil was obtained. Then percentage of water holding capacity of soil was calculated.

(2) Pretreatment of the soil sample: Each soil sample (80 g) was weighed in a 100 ml plastic container and mixed with deionized water to 100% maximum water holding capacity (MWHC). Care was taken to make sure that there was no excess water on the soil surface. The soils were equilibrated for 24 hours, loosely covering the container with a plastic plate or sheet to minimize evaporation. After 24 hours of hydration, the soil sample was evenly divided into three separate small weigh dishes ready for deployment.

(3) DGT deployment: The assembled DGT devices were gently placed on the soil surface of each dish for 24 hours, but the gel films were not squeezed. The containers were closed, and weigh dishes with wet cellulose were placed in the containers to retain the soil moisture. Three weigh dishes were kept at room temperature for 24 hours.

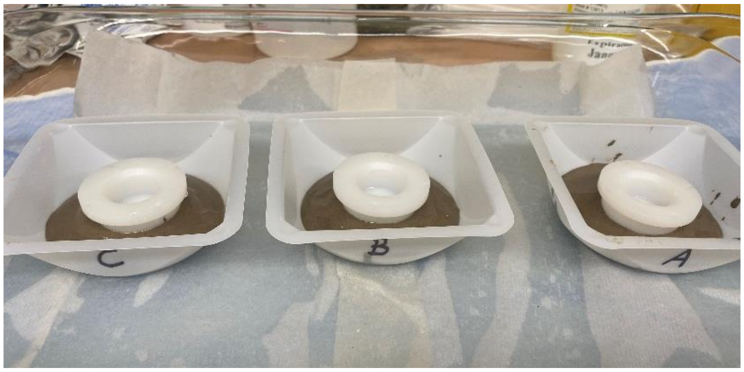


Figure 3.2 DGT deployment

(4) DGT retrieval and elution: After 24 h, all of the DGT devices were retrieved and rinsed with deionized water. The binding gel layers were removed from the DGT units, placed in polyethylene vials, and eluted in 1 mL of 1 mol/L HNO_3 for 24 h. The Cd, Cu, Fe, Mn, Ni, Zn, Ca and Mg concentrations in the extractant were determined by ICP-OES or ICP-MS in samples that were below the Cd MDL of ICP-OES (0.0002 mg/L Cd).

The amount (M) of Cd accumulated on resin gel was calculated using the formula

$$M = C\,(V_e + V_g)\,/\,f_e \text{ ———— } (1)$$

Where C is the concentration of Cd in the 1 M HNO_3 extract, V_e the volume of the 1 M HNO_3 extract, V_g the volume of the resin gel, and f_e the elution factor (Zhang et al., 1998) accounting for incomplete elution. Using M, the DGT-available Cd (Cd_{DGT}) concentration was calculated using $C_{DGT} = M\Delta g/DAt$.

Soil: one sample from each site was used for DGT analysis, which was performed on three replicate subsamples.

Results and Discussion

Extracted metal concentrations in Idaho soils

Table 3.2 presents the total metal concentrations in soil in different sampling sites. Tables 3.3 and 3.4 show the fraction of total metals used to determine the bioavailable fraction for the plant uptake. The bioavailability of trace metals in a given soil depends on both their concentrations in the soil solution and their rate of transportation through the soil (Hooda et al., 1999). However, the data in this study illustrates that the metal concentrations that can be extracted by the DTPA method were significantly higher than the extracted concentrations from the DGT method. According to this study, the order of total metal concentration was Mn>Zn> Cu>Fe>Cd in each site. Soda Springs soils contained the highest amount of Cd in soils and it was considerably high compared to the other regions. Nunes et al. (2014) found that the total metal varies according to the order of Cr>Zn>Ni>Pb>Cu>>Cd in non-contaminated Mediterranean agricultural soils. Their DTPA bioavailable Cd, Cu and Zn concentrations in soils varied from 0.04 to 0.90 mg/kg, 0.10 to 6.30 mg/kg, and 0.18 to 2.50 mg/kg respectively. Their range of DTPA metal variations in soils in this study is very similar to our study (Table 3.2).

DGT technique is used to measure free and easily dissociated metal species that are bioavailable for plant uptake (Davison & Zhang, 1994). The detected concentrations of metals using the DGT method were lower than the DTPA method in our study (Table 3.4). Among the sampling sites, the lowest amount of Cd_{DGT} was reported in Rupert (~0.00004 mg/L) and Soda Springs soils contained the highest amount of average Cd_{DGT} concentration (0.00122 mg/L) which was 30 times greater than the lowest concentration (Figure 3.3). The other sites also had a lower amount of Cd than the Soda Springs site. However, the average Cd_{DGT} varied from 3.81×10^{-5} to 1.22×10^{-3} mg/L across the eight different sites. A study conducted in China found that Cd in soil using the DGT method ranged from 9.16×10^{-3} to 49.7×10^{-3} mg/L (Ningning et al., 2015). Additionally, the average soil DGT Cd concentration in organic farmland in Southern China was reported as 0.005 mg/L (Williams et al., 2012). Compared with these studies, the bioavailable Cd from the DGT method in this study is significantly low.

Nowel et al. (2004) found that the average DGT Cu and Zn concentrations in Switzerland contaminated fields were 0.262 and 4.160 mg/L, respectively and Zn was rapidly available in that field soil. The measured Cu and Zn concentrations from the DGT method in this study are very low.

Table 3.2 Summary statistics of total soil metal concentrations in soils in Idaho

	Total Cd (mg/L)		Total Cu (mg/L)		Total Fe (%)		Total Mn (mg/L)		Total Zn (mg/L)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Aberdeen	0.430	1.00 E-02	15.4	0.650	1.77	3.00 E-02	3.97 E02	6.64	64.9	1.33
Soda spring	1.14	2.00 E-02	22.7	0.460	2.27	1.00 E-02	6.82 E02	5.59	90.2	1.06
Ashton	0.510	1.00 E-02	19.6	0.740	2.58	3.00 E-02	6.25 E02	6.50	84.8	0.900
Rupert	0.490	1.00 E-02	19.7	0.970	2.14	2.00 E-02	5.33 E02	5.12	79.4	2.15
Kimberly	0.560	1.00 E-02	21.3	0.330	2.42	1.00 E-02	6.60 E02	8.42	1.01 E02	1.53
Moscow	0.160	1.00 E-02	19.7	0.170	2.74	1.00 E-02	6.48 E02	13.8	65.92	0.400
Tammany	0.140	0.00	21.7	0.140	3.06	2.00 E-02	6.59 E02	4.91	66.0	0.620
Tensed	0.130	1.00 E-02	21.9	0.150	3.08	0.02	6.49 E02	12.2	72.9	1.20

Table 3.3 Summary statistics of DTPA extractable concentration of metals in soil in Idaho

	DTPA Cd (mg/L)		DTPA Cu (mg/L)		DTPA Fe (mg/L)		DTPA Mn (mg/L)		DTPA Zn (mg/L)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Aberdeen	9.00 E-02	0.00	0.850	3.00 E-02	5.08	0.180	6.48	0.220	1.92	1.75
Soda spring	0.460	2.00 E-02	1.70	5.00 E-02	64.0	5.28	45.5	3.11	2.56	0.100
Ashton	0.200	1.00 E-02	0.880	3.00 E-02	64.5	7.22	22.5	3.74	2.31	0.110
Rupert	0.130	0.00	1.20	2.00 E-02	7.93	0.170	13.5	0.520	2.31	0.190
Kimberly	0.150	1.00 E-02	1.35	1.00 E-02	10.5	0.260	14.4	0.710	4.10	0.130
Moscow	1.00 E-02	0.00	1.93	3.00 E-02	1.24 E02	1.87	48.8	2.59	1.20	2.00 E-02

Tammany	7.00 E-02	0.00	0.950	1.00 E-02	63.1	0.970	37.7	3.74	0.800	5.00 E-02
Tensed	9.00 E-02	0.00	1.32	2.00 E-02	1.37 E02	7.05	33.8	0.740	1.78	0.190

Table 3.4 Summary statistics of the calculated DGT concentration of metals using the DGT equation (Equation 1). RSD is the relative standard deviation of replicates.

	Cd_{DGT} (mg/L)		Cu_{DGT} (mg/L)		Fe_{DGT} (mg/L)		Mn_{DGT} (mg/L)		Zn_{DGT} (mg/L)	
	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
Aberdeen	3.93E-05	25.7	6.74E-04	10.9	3.00 E-03	54.4	5.00 E-02	7.99	3.60E-03	36.9
Soda spring	1.22E-03	6.46	5.63E-04	10.8	1.70E-02	85.6	0.420	6.39	4.19E-03	9.79
Ashton	1.30E-04	19.2	7.94E-04	64.9	6.00 E-02	13.3 E01	0.120	32.9	3.10E-03	21.3
Rupert	3.81E-05	24.8	1.034E-03	7.82	8.47E-03	11.7	0.320	5.12	2.22E-03	17.7
Kimberly	1.05E-04	30.6	8.33E-04	13.5	2.00 E-02	87.7	0.170	12.0	2.71E-03	25.0
Moscow	3.23E-04	7.15	4.26E-04	7.43	4.94E-03	8.14	0.370	2.92	3.94E-03	23.1
Tammany	1.56E-04	38.6	1.26E-03	54.0	0.190	11.1 E01	0.310	39.1	3.81E-03	34.8
Tensed	1.45E-04	18.1	8.60E-04	37.1	4.00 E-02	11.0 E01	0.290	15.5	4.79E-03	13.9

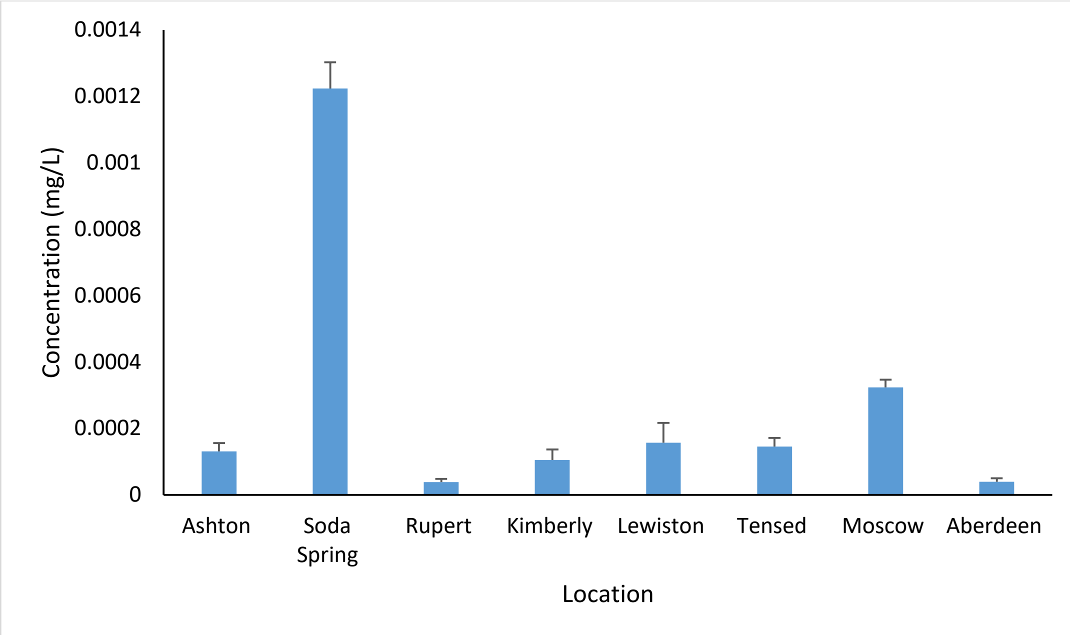


Figure 3.3 The calculated CdDGT concentrations measured in the soil in different sampling sites (Bars show the standard error in mean of lab sub-samples)

Correlations between extraction methods and grain Cd

Since the dataset was small and contained an outlier, the Spearman correlation test was applied. Table 3.5 presents the Spearman correlation coefficients of grain Cd and extractable soil Cd concentrations. Total Cd and DTPA Cd are positively correlated with grain Cd. Total Cd and DTPA Cd are strongly correlated with grain Cd (Figure 3.4).

Chapter 2 showed that total Cd and DTPA Cd are best soil variables to predict the Cd uptake by plants. Wu et al. (2021) also found DTPA extractable Cd is a better predictor ($p<0.001$) of Cd transportation in the soil-rice system. Not only that, Khanmirzaei et al. (2013) reported that DTPA extractable Cd from highly carbonated soils in Iran was a good predictor to predict the Cd phytoavailability in Durum Wheat.

Cornu and Denaix (2006) showed that plant Cd concentration was weakly related to the DGT-based Cd concentrations in lettuce and mentioned further studies are needed. The study conducted by Oporto et al. (2008) found that DGT may fail to predict metal uptake by plants at a high metal concentration at which the plant uptake becomes saturated because the Cd uptake by the plant is not limited by diffusion. Perez and Anderson (2009) found that direct measurement of Cd (Cd_{DGT}) correlated better with Cd in edible plant tissue, but the effective concentration (Cd_{CE}) calculated from DGT did not correlate ($R= 0.45$) with Cd in wheat grain and potatoes. Luo et al. (2014) suggested that Cd uptake by radish is not simply related with the diffusional supply from soil solution augmented by resupply from the solid phase because the effective concentration from the DGT method was poorly correlated (r^2= 0.58) with Cd uptake by radish. Thus, the lack of correlation reported in the literature and the current research indicate that DGT method may not be a good method to estimate Cd accumulation in crops, and it may be plant species specific.

However, many studies have identified the DGT technique as a promising tool to assess Cd biological effectiveness because more elements in the soil solid phase desorb and diffusive through the DGT surface is similar to uptake of an element by the plant (Bade et al., 2012; Guan et al., 2016; Luo et al., 2014). Nolan et al. (2005) found that DGT measurements in soil were effective in predicting plant Cd accumulation in wheat from contaminated soils. Additionally, Tian et al. (2007) reported that DGT Cd is a good predictor of Cd concentrations in roots and grains of rice even at low concentrations.

Table 3.5 Spearman correlation of extractable concentrations from soil, grain Cd, and BAF.

	Grain Cd	DGT Cd	DGT Cu	DGT Fe	DGT Mn	DGT Zn	DTPA Cd	DTPA Cu	DTPA Fe	DTPA Mn	DTPA Zn	Total Cd
Grain Cd	1											
DGT Cd	-0.361	1										
DGT Cu	-0.301	-0. 405	1									
DGT Fe	-0.145	0. 238	0.595*	1								
DGT Mn	0.108	0. 619	-0.166	-0.0952	1							
DGT Zn	-0.000	0. 785*	-0.309	0.0952	0.357	1						
DTPA Cd	0.627*	0.238	-0.285	0.166	0.357	-0.143	1					
DTPA Cu	0.120	0. 428	-0.333	-0.381	0.881*	0.238	0.405	1				
DTPA Fe	-0.0843	0.809*	-0.167	0.333	0.524	0.8333*	0.119	0.357	1			
DTPA Mn	-0.0482	0.857 *	-0.262	0.0952	0.833*	0.714*	0.286	0.762**	0.833	1		
DTPA Zn	0.494	-0.119	-0.119	-0.0238	0.190	-0.309	0.833*	0.452	-0.0952	0.143	1	
Total Cd	0.566*	-0.0476	-0.357	-0.143	-0.0952	-0.405	0.810*	0.286	-0.381	-0.0476	0.786*	1
Cd BAF	0.265	0.0476	0.238	0.333	0.0952	0.238	0.238	-0.167	0.476	0.0714	-0.167	-0.476

*p<0.05

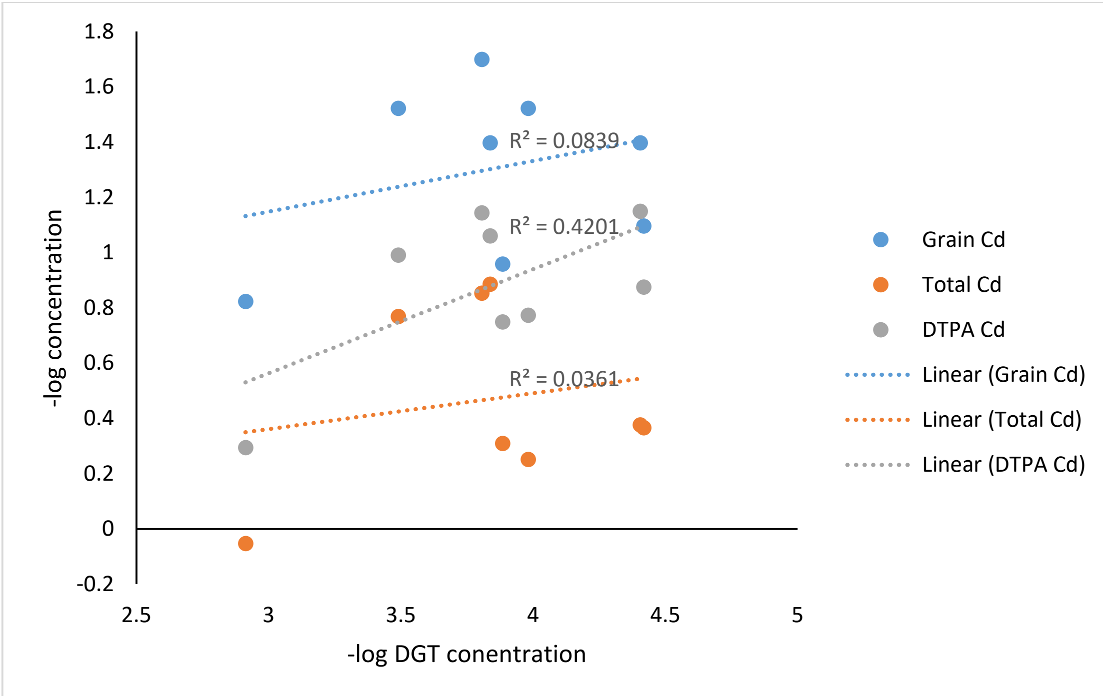


Figure 3.4 The linear relationship between minus log transformation of DGT Cd concentration in soil vs minus log transformation of DTPA extraction, total Cd in soil, and grain Cd concentration

Conclusion

The bioavailability of Cd in the soil is an important determinant to predict Cd uptake by wheat. Several extraction methods and techniques have been identified to measure Cd bioavailability in the soil. Among those, the DTPA extraction method and DGT technique were used in this study. The highest bioavailable Cd was found in the Soda Springs soils and the lowest was reported in Tensed soils from both DTPA and DGT methods. While the average total Cd, DTPA Cd, and DGT Cd are positively correlated with Grain Cd, strong correlations are observed only with total Cd and DTPA Cd (Table 3.4). Among DTPA and total Cd, DTPA is the best predictor for Cd uptake by wheat in this study.

Compared to the DTPA extraction method, very low concentrations in soils were observed in the DGT method. Although DGT is known to be the best predictor to predict Cd in crops over the traditional extraction methods, it was not an effective method in this study.

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Appendix

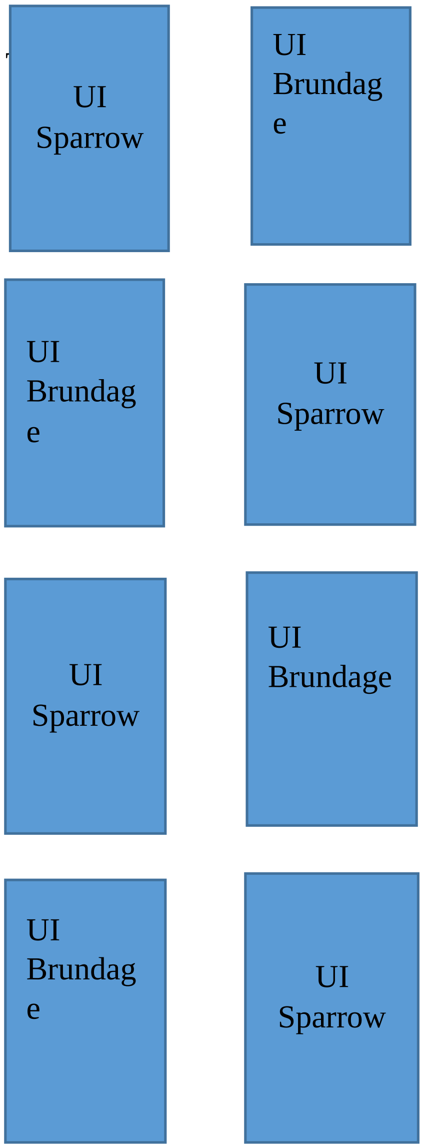

Figure 2.14 Example of plot layout structure showing wheat variety in randomized block design for the Tammany site.

Figure 2.15 Photos showing soil sampling.

Table 2.12 Example table of data showing soil pH and total grain Cd for Tammany.

Site	Variety	Replicate*	Soil pH	Total Cd in grain (mg/kg)
Tammany	UI Sparrow	1	5.55	0.03
		2	5.57	0.02
		3	5.85	0.03
		4	5.58	0.02
	UI Brundage	1	5.47	0.07
		2	5.31	0.06
		3	5.46	0.06
		4	5.52	0.06

*plots arranged in randomized complete block design (see Figure 2.1).

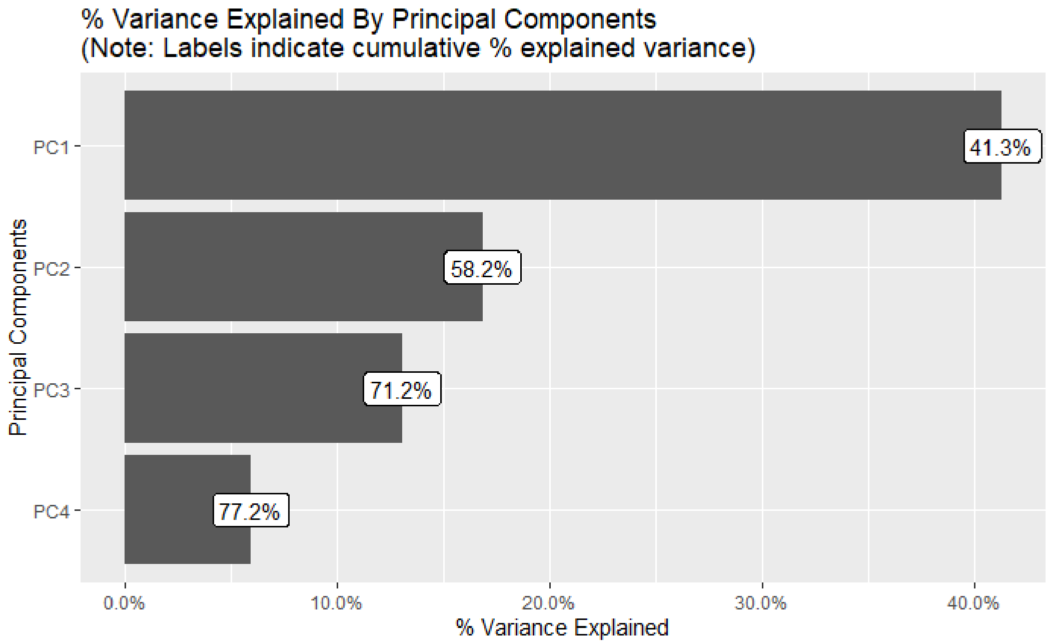

Figure 2.16 Bar graph of Principal components vs % variance of soil variables

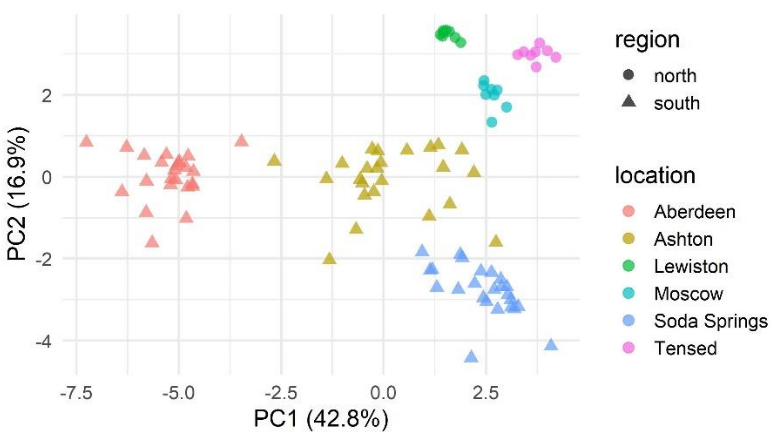

Figure 2.17 Scores for observations by region and location

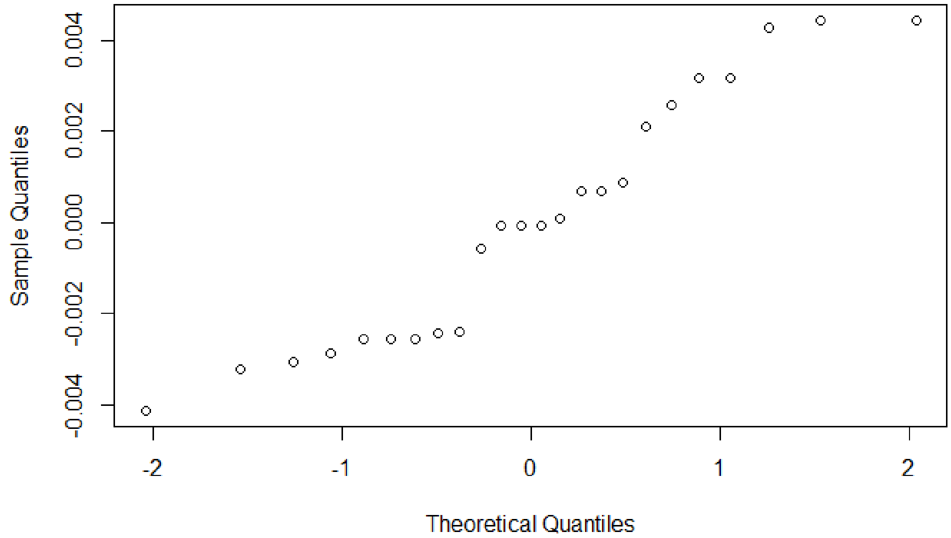

Figure 2.18 Normal Q-Q plot for the model of northern Idaho

Linear relationship between soil variables

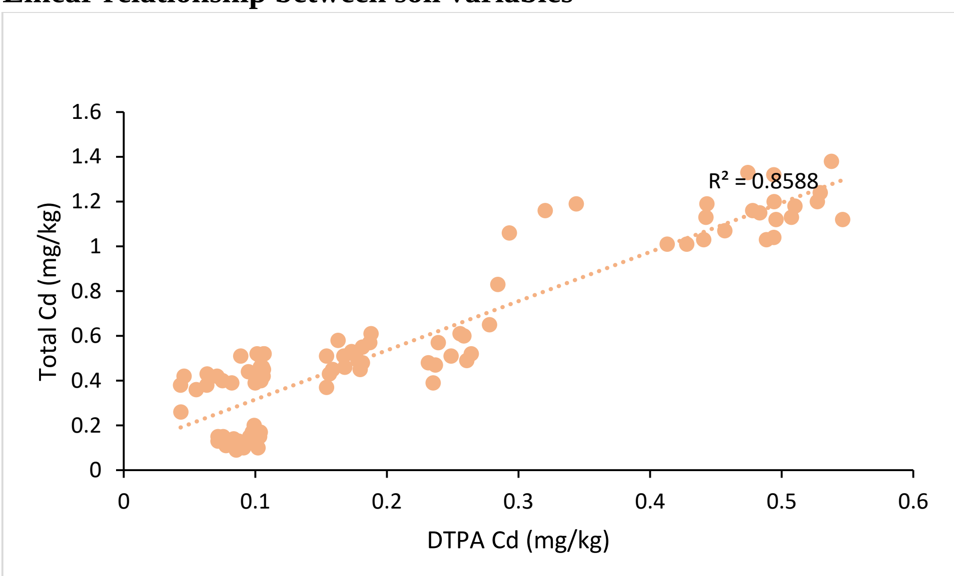

Figure 2.19 Total Cd vs DTPA Cd in Idaho soils

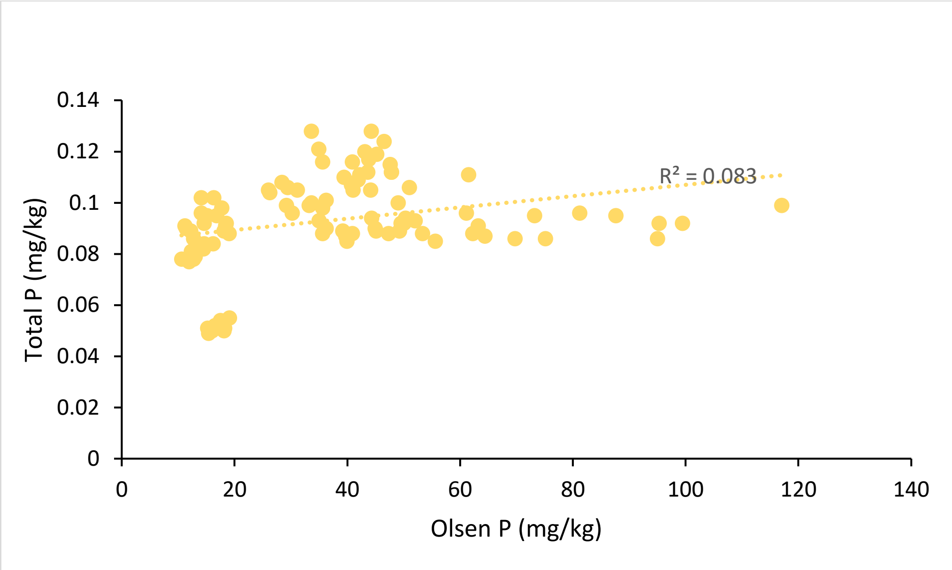

Figure 2.20 Total P vs Olsen P in Idaho soils

Table 2.13 Pairwise comparison of variety and locations on northern Idaho

Location	**Variety**	**Contrast**	**Estimated regression slope**	**df**	**t.ratio**	**p.value**
Tammany		Brundage - UI Sparrow	0.0375 (0.00264)	9.00	14.2	<0.000100
Moscow		Brundage - UI Sparrow	0.0450 (0.00264)	9.00	17.1	<0.000100
Tensed		Brundage - UI Sparrow	0.0175 (0.00264)	9.00	6.64	0.000600
	Brundage	Tammany - Moscow	-0.0100 (0.00358)	10.3	-2.79	0.0989
	Brundage	Tammany - Tensed	0.0300 (0.00358)	10.3	8.37	<0.000100
	Brundage	Moscow - Tensed	0.0400 (0.00358)	10.3	11.160	<0.0001
	UI Sparrow	Tammany - Moscow	-0.0025 (0.00358)	10.3	-0.697	0.9433
	UI Sparrow	Tammany - Tensed	0.0100 (0.00358)	10.3	2.790	0.0989
	UI Sparrow	Moscow - Tensed	0.0125 (0.00358)	10.3	3.487	0.0329

(Values in parenthesis are standard error)

Table 2.14 Concentrations of soil properties in sampling sites, Idaho.

Site	**pH**		**Organic Carbon**		**CEC (mmol(+)/kg)**		**Total Cd (mg/kg)**		**Total P (ppm)**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Aberdeen	8.18	2.00 E-02	0.760	3.00 E-02	268	0.230	0.430	1.00 E-02	0.0900	0.00
Soda Spring	6.03	0.140	1.25	4.00 E-02	168	0.230	1.14	2.00 E-02	0.110	0.00
Ashton	6.69	0.210	1.59	3.00 E-02	180	0.720	0.510	1.00 E-02	0.0900	0.00
Moscow	4.90	3.00 E-02	1.90	3.00 E-02	169	0.840	0.160	0.00	0.0900	0.00
Tammany	5.54	5.00 E-02	1.60	2.00 E-02	152	0.490	0.140	0.00	0.0500	0.00
Tensed	5.05	6.00 E-02	2.73	9.00 E-02	171	0.910	0.130	1.00 E-02	0.100	0.00
	Chloride (ppm)		**NO_3^--N (ppm)**		**NH_4^+-N (ppm)**		**Total Zn (ppm)**		**Olsen P (ppm)**	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Aberdeen	16.3	0.930	1.32	0.120	4.58	0.140	64.9	1.33	14.6	0.520
Soda Spring	5.78	2.80	10.6	1.98	14.3	1.54	90.2	1.06	42.5	1.32
Ashton	11.0	1.21	25.8	7.68	6.47	0.370	84.8	0.900	63.3	4.70
Moscow	4.18	0.390	2.71	0.320	10.3	1.58	65.9	0.400	47.9	2.34
Tammany	4.74	0.400	0.820	9.00 E-02	3.36	0.400	66.0	0.620	17.0	0.510
Tensed	3.91	0.810	4.90	0.610	8.72	0.840	72.9	1.20	29.5	0.990

Table 2.15 Bioaccumulation factor in each variety.

Region	Variety	Bioaccumulation factor	
		Mean	SE
Southern Idaho	UI Platinum	0.148	0.0222
	LCS Star	0.157	0.0169
	CdDH-016	0.136	0.0258
	CdDH-026	0.145	0.0181
	CdDH-028	0.134	0.0190
	CdDH-266	0.138	0.0205
Northern Idaho	Brundage	0.397	0.0342
	UI Sparrow	0.161	0.0154

Table 2.16 The correlation between Grain Cd and soil properties

	Grain Cd	*pH*	*DTPA Cd*	*DTPA Zn*	*Total Cd*	*Total Zn*	*Total P*	*Olsen P*	*Organic Carbon*	*Total N*	*NO3-N*
Grain Cd	1										
pH	-0.08624	1									
DTPA Cd	0.665236	-0.31368	1								
DTPA Zn	0.210975	0.05897	0.323185	1							
Total Cd	0.589227	0.009798	0.92669	0.351667	1						
Total Zn	0.649195	-0.22314	0.77483	0.550412	0.724003	1					
Total P	0.328007	-0.05489	0.659001	0.370588	0.69251	0.627834	1				
Olsen P	0.61502	-0.14949	0.320338	0.098745	0.241013	0.550086	0.28806	1			
Organic Carbon	-0.1678	-0.67948	-0.11661	-0.05302	-0.35627	0.139161	0.033882	0.288865	1		
Total N	0.071954	-0.65063	0.07091	0.022978	-0.16653	0.365855	0.193532	0.476776	0.951279	1	
NO3-N	0.428829	-0.1458	0.185084	0.147087	0.152229	0.361955	0.104058	0.379918	0.09911	0.227159	1

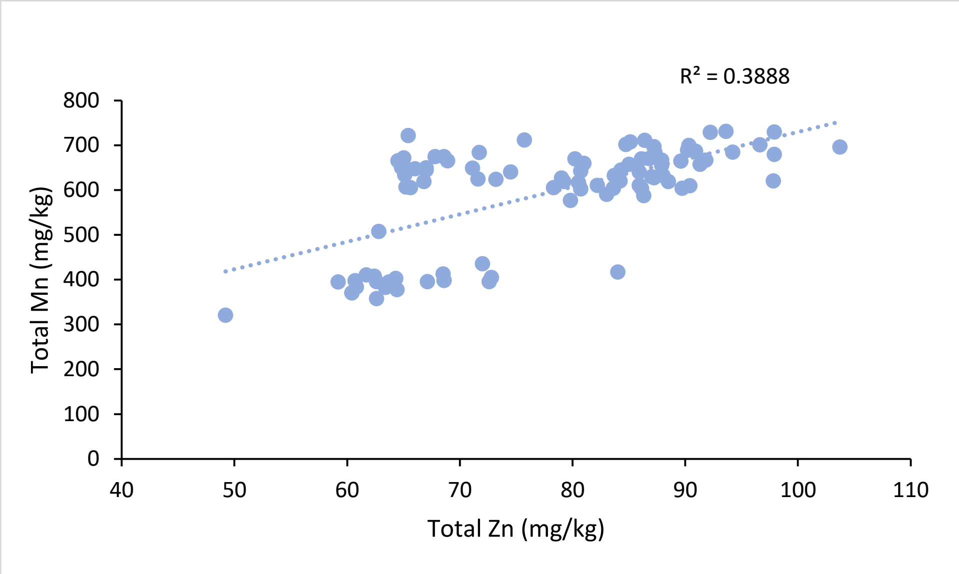

Figure 2.21 Total Mn vs Total Zn in Idaho soils

Table 2.17 The significant difference of soil variable by regions of southern and northern Idaho using ANOVA

Soil Factor	**Mean squared**	**P - value**
pH	54.57692	1.21E-10
DTPA Cd	0.45839	7.33E-06
Total Cd	5.384882	2.64E-12
Total P	0.004961	2.27E-05
Olsen P	1826.239	0.0558
CEC	282.5722	0.000325
Total N	3579143	1.89E-06
Organic C	13.52838	1.05E-13
NO3-N	1700.075	0.056831
NH4-N	22.02363	0.416186
Cl^-	766.4454	0.001941

Table 2.18 Pairwise comparison of soil properties and location

Location	pH	DTPA Cd	Total Cd	Total P	Olsen P	CEC
	P value					
Ashton-Aberdeen	0.0000000	0.0000000	0.0057525	0.7132130	0.0000000	0.0000000
Tammany-Aberdeen	0.0000000	0.9829688	0.0000000	0.0000000	0.9968022	0.0000000

Moscow-Aberdeen	0.0000000	0.9836054	0.0000000	0.9829266	0.0000000	0.0000000
Soda Springs-Aberdeen	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000	0.0000000
Tensed-Aberdeen	0.0000000	0.9992993	0.0000000	0.0000048	0.0433761	0.0000000
Tammany-Ashton	0.0004113	0.0000000	0.0000000	0.0000000	0.0000000	0.0328293
Moscow-Ashton	0.0000000	0.0000024	0.0000000	0.9995488	0.0332766	0.8537433
Soda Springs-Ashton -	0.0074422	0.0000000	0.0000000	0.0000000	0.0000012	0.5180899
Tensed-Ashton	0.0000002	0.0000006	0.0000000	0.0002752	0.0000000	0.9221782
Location	Total N	Organic C	NO3-N	NH4-N	Cl^-	
	P value					
Ashton-Aberdeen	0.0000000	0.0000000	0.0005781	0.6259036	0.1873334	
Tammany-Aberdeen	0.0000000	0.0000000	0.9999999	0.9796809	0.0071752	
Moscow-Aberdeen	0.0000000	0.0000000	0.9999776	0.0148037	0.0040829	
Soda Springs-Aberdeen	0.0000000	0.0000000	0.5745076	0.0000000	0.0001910	
Tensed-Aberdeen	0.0000000	0.0000000	0.9976837	0.1618269	0.0031153	
Tammany-Ashton	0.0000082	0.9999992	0.0291867	0.4590741	0.3967120	
Moscow-Ashton	0.9997303	0.0003237	0.0550505	0.2299133	0.2998431	
Soda Springs-Ashton -	0.0000000	0.0000000	0.0913590	0.0000001	0.2209345	
Tensed-Ashton	0.0000000	0.0000000	0.1075003	0.7777074	0.2597737	

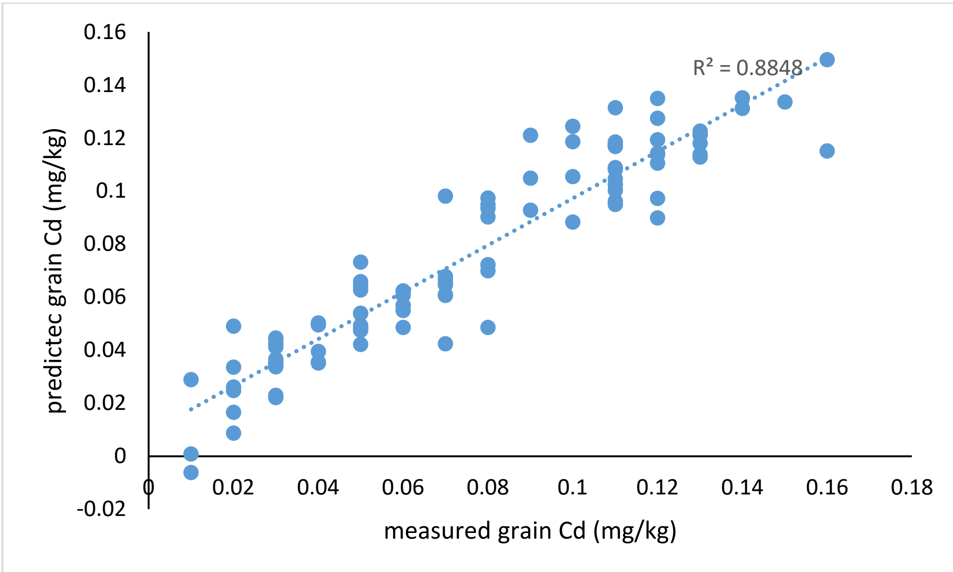

Figure 2. 22 Predicted grain Cd vs measured grain Cd in stepwise regression model with variety (R^2 = 0.88).

Table 2.19 The mean squared values and node purity values of variables for Random Forest model.

variable	%Inc MSE	Inc Node Purity
DTPA Cd	7.39e-04	0.0348
Total Zn	4.98e-04	0.0286
Olsen P	2.84e-04	0.0168
DTPA Fe	1.65e-04	0.00614
Total Cd	1.19e-04	0.00939
Total Fe	8.02e-05	0.00480
DTPA Zn	8.01e-05	0.00658
NO3_N	7.39e-05	0.00773
Region	5.84e-05	0.00205
pH	2.97e-05	0.00207
DTPA Mn	2.48e-05	0.00164
Total N	2.13e-05	0.00171
Total Mn	2.01e-05	0.00173

K	1.80e-05	0.00331
Organic carbon	1.69e-05	0.00155
Total Cu	1.55e-05	0.00168
SO_4^{2-} S	1.54e-05	0.00195
CEC	1.50e-05	0.00133
DTPA Cu	1.48e-05	0.00135
NH_4^+ N	1.08e-05	0.00166
Silt	6.07e-06	0.00122
Sand	6.03e-06	0.000799
Total P	5.15e-06	0.00278
EC	2.96e-06	0.00137
Clay	7.00e-07	0.000523
Cl	-2.39e-06	0.00151

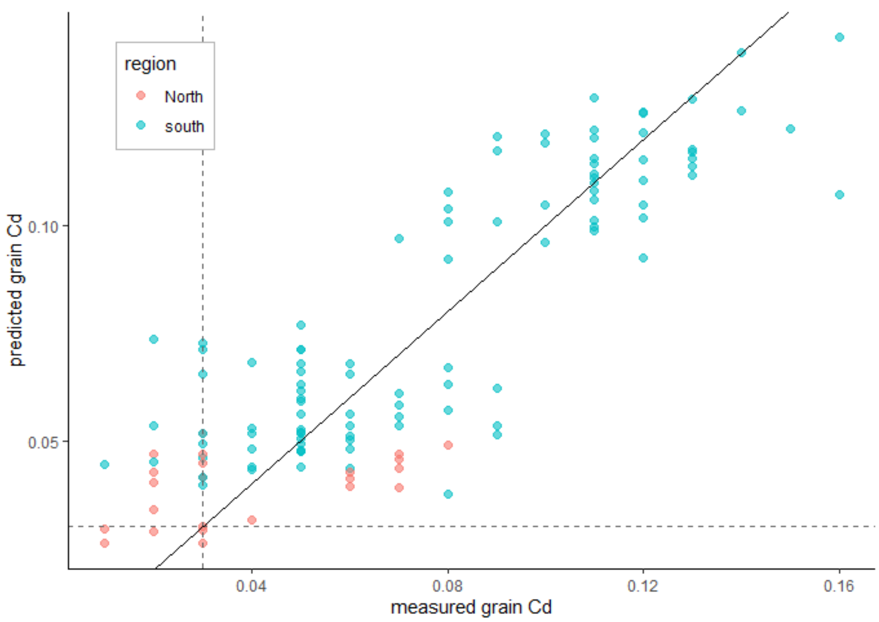

Figure 2.23 Predicted grain Cd vs measured grain Cd in stepwise regression model validation for new dataset. (R^2 = 0.74)

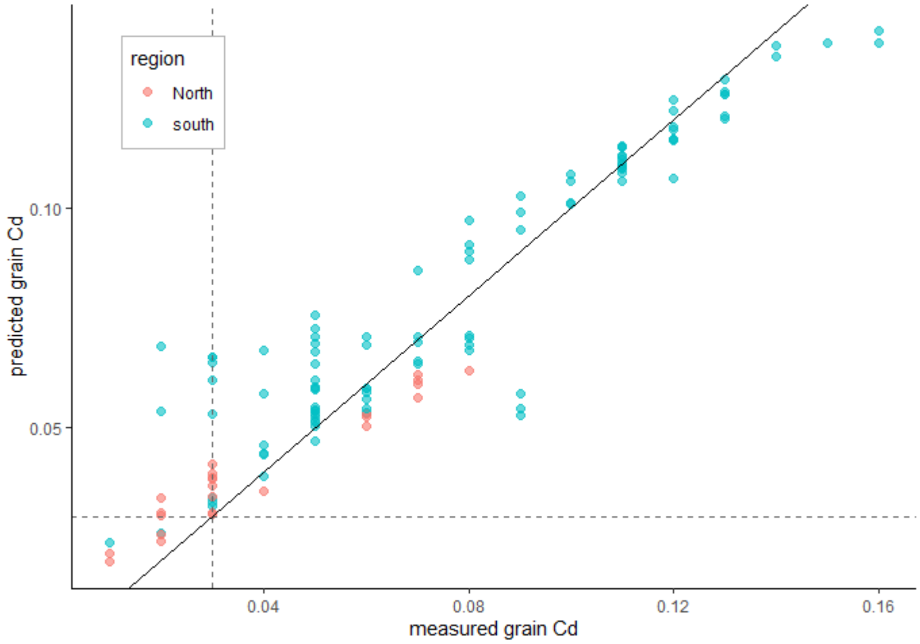

Figure 2.24 Predicted grain Cd vs measured grain Cd in Random Forest model validation for new dataset which includes base data set and Rupert and Kimberly data ($R^2 = 0.88$)

Table 2. 20 Summary output of the stepwise regression to predict significant soil properties (among 25 variables) that affect the grain Cd concentration in Southern Idaho region.

	Variable importance	Estimate	Std. Error	Pr(>|t|)
(Intercept)	0.410	2.153e-02	5.245e-02	0.6828
Olsen P	7.247	9.418e-04	1.300e-04	6.84e-10 ***
Total Cd	-5.496	-9.981e-02	1.816e-02	7.23e-07 ***
DTPA Cu	4.458	8.046e-02	1.805e-02	3.41e-05 ***
Total Mn	2.002	9.420e-05	4.706e-05	0.0496 *
NO_3_N	1.958	1.814e-04	9.266e-05	0.0546 .
Organic Carbon	-1.231	-1.484e-02	1.206e-02	0.2229
pH	-1.019	-4.523e-03	4.441e-03	0.3123

Residual standard error: 0.0157 on 64 degrees of freedom
Multiple R-squared: 0.8332, Adjusted R-squared: 0.815
F-statistic: 45.67 on 7 and 64 DF, p-value: < 2.2e-16

Table 2.21 Type III analysis of variance table for the effect of variety and location to the grain Cd concentrations in southern Idaho only for LCS star and UI Sparrow varieties

	Sum of squared	**Mean squared**	**Degree of freedom**	**F value**	**Pr(>F)**
location	0.0188	0.00940	2	42.3	6.80e-07 ***
variety	0.00135	0.00135	1	6.07	0.0263 *
Location*variety	0.000675	0.0003375	2	1.52	0.251

Table 3.6 Method detection limit for DGT method calculation

Sample ID	concentration (ppb)	Intensity	Blank ID	**Intensity**
1	40	4178.5011	1	19.7714
2	40	4179.3556	2	15.6021
3	40	4193.8907	3	12.2032
4	40	4167.8346	4	20.532
5	40	4179.6581	5	12.73
6	40	4170.8035	6	19.9484
7	40	4185.4511	7	18.9458
8	40	4176.4117	8	21.4667
			mean (Yblank)	17.64995

std (S) = 8.130235079
Slop of Calibration curve=105407.1975
Student t value = 2.998
Ydl = Yblank + (t*S)
Ydl (Signal detection limit)   42.02439477
Concentration detection limit = (t*S)/m
Concentration detection limit=0.000231241

Table 3.7 Measured DGT data using IPC-OES

Sample	Ca (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
Ashton A	10.32472	0.000133	0.000678	0.01543	0.378604	0.111648	0.000696	0.003167
Ashton B	10.37151	0.000158	0.000588	0.016346	0.379354	0.123621	0.000779	0.0035
Ashton C	14.35846	0.000195	0.001767	0.175157	0.553019	0.199538	0.001557	0.0047
Soda Spring A	6.585125	0.001554	0.000642	0.011967	1.033829	0.525567	0.00195	0.005154

Soda Spring B	6.057683	0.0016	0.000713	0.042479	0.932	0.521225	0.002363	0.005746
Soda Spring C	5.507015	0.001412	0.000796	0.009723	0.833915	0.467627	0.002031	0.004731
Rupert A	9.727	4.32E-05	0.001373	0.010279	1.625357	0.376408	0.001553	0.002315
Rupert B	7.640885	3.85E-05	0.0012	0.0095	1.225692	0.36266	0.001446	0.002676
Rupert C	10.10196	6.08E-05	0.001384	0.011928	1.68124	0.401084	0.001664	0.003281
Kimberly A	9.179819	0.000108	0.001223	0.043442	1.254354	0.228231	0.001	0.004031
Kimberly B	8.094931	0.000107	0.000952	0.008821	1.098345	0.189506	0.001018	0.002418
Kimberly C	9.15063	0.000176	0.001005	0.012667	1.269074	0.184093	0.001143	0.003649
Tammany A	6.409887	0.000278	0.002596	0.547491	1.146357	0.53527	0.014922	0.006626
Tammany B	4.94052	0.000172	0.001268	0.08638	0.894128	0.2987	0.002188	0.00396
Tammany C	4.893011	0.000133	0.000963	0.083115	0.892059	0.27377	0.00167	0.003615
Tensed A	6.825982	0.000218	0.001545	0.121755	0.7035	0.408877	0.005432	0.006914
Tensed B	5.368258	0.000167	0.000758	0.016275	0.554938	0.321546	0.001933	0.005479
Tensed C	5.019704	0.000158	0.000981	0.022362	0.516173	0.310488	0.001969	0.005473
Moscow A	5.967088	0.000436	0.000528	0.006336	0.7787	0.453436	0.004604	0.005
Moscow B	4.89058	0.000384	0.000512	0.006572	0.632312	0.428432	0.005776	0.005956
Moscow C	5.477731	0.000388	0.000588	0.005608	0.714338	0.446719	0.004312	0.003708

Table 3.8 Calculated DGT concentrations

Sample	Ca (mg/kg)	Cd (mg/kg)	Cu (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Mn (mg/kg)	Ni (mg/kg)	Zn (mg/kg)
Ashton A	8.2974	0.000107	0.000532	0.012359	0.304263	0.093406	0.000591	0.002549
Ashton B	8.335005	0.000127	0.000462	0.013093	0.304866	0.103423	0.000661	0.002817
Ashton C	11.53909	0.000157	0.001388	0.140303	0.444431	0.166936	0.001321	0.003783
Soda Spring A	5.292097	0.001249	0.000504	0.009585	0.830831	0.439697	0.001654	0.004149
Soda Spring B	4.868222	0.001286	0.00056	0.034026	0.748996	0.436064	0.002004	0.004625
Soda Spring C	4.425681	0.001134	0.000625	0.007788	0.670171	0.391223	0.001723	0.003808
Rupert A	7.817047	3.47E-05	0.001079	0.008233	1.306209	0.314908	0.001317	0.001864
Rupert B	6.140552	3.09E-05	0.000943	0.00761	0.98502	0.303406	0.001226	0.002154
Rupert C	8.118381	4.89E-05	0.001088	0.009554	1.351119	0.335553	0.001411	0.002641
Kimberly A	7.377308	8.65E-05	0.000961	0.034798	1.008054	0.190941	0.000848	0.003245
Kimberly B	6.505444	8.56E-05	0.000748	0.007065	0.882678	0.158544	0.000864	0.001947

Kimberly C	7.35385	0.000141	0.000789	0.010146	1.019884	0.154015	0.000969	0.002937
Tammany A	5.151268	0.000224	0.002039	0.438548	0.921263	0.447814	0.012657	0.005334
Tammany B	3.97042	0.000138	0.000996	0.069192	0.718561	0.249897	0.001856	0.003188
Tammany C	3.93224	0.000107	0.000756	0.066576	0.716898	0.22904	0.001417	0.00291
Tensed A	5.48566	0.000175	0.001214	0.097527	0.565364	0.342073	0.004607	0.005565
Tensed B	4.314169	0.000134	0.000596	0.013036	0.445972	0.26901	0.00164	0.004411
Tensed C	4.034056	0.000127	0.00077	0.017912	0.414819	0.259759	0.00167	0.004406
Moscow A	4.795415	0.00035	0.000415	0.005075	0.625798	0.379351	0.003905	0.004025
Moscow B	3.930286	0.000309	0.000402	0.005264	0.508154	0.358432	0.004899	0.004794
Moscow C	4.402146	0.000312	0.000462	0.004492	0.574074	0.373732	0.003657	0.002985