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## The dietary mineral content of *Claytonia lanceolata* corms from multiple populations across the Columbia Plateau region of North America

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**The Dietary Mineral Content of *Claytonia lanceolata* Corms from Multiple  
Populations across the Columbia Plateau Region of North America**

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A Thesis  
Presented to  
Eastern Washington University  
Cheney, WA

In partial fulfillment of the Requirements  
for the Degree  
Master of Science in Biology

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**By**  
**Paul Reilly**  
**Spring 2015**

**Thesis of Paul Reilly Approved by**

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**Date**

## Abstract

Since divergence from our ancestral lineage ~2.8 mya, humans have relied on foraged foods to obtain dietary mineral nutrition. Around 12,000 years ago, people began shifting towards a lifestyle of food production. The rapid shift in lifestyle significantly altered the human diet, and research suggests our genomes have had insufficient time to adjust. Due to the discord between genome and environment, unprecedented levels of disease and malnutrition are epidemic in certain subsets of the population. Studies suggest the most cost effective way to mitigate the risk of these diseases is to find ways to increase micronutrient consumption. As a class, wild plant foods appear to be more nutrient dense than most modern foods, so including or reintroducing wild foods into the human diet should provide a number of health benefits. Unfortunately, little is known about the dietary mineral composition of many foraged food plants and even less is known about potential differences in nutrient composition between populations or across the phenology. The Western Spring Beauty (*Claytonia lanceolata*) is no exception, whereas some information from a proximate analysis conducted in 1938 exists, data about micronutrient composition is altogether lacking. Indigenous people harvested the corms (underground stems) of *C. lanceolata* since prehistory to eat immediately, store for delayed consumption, or use as a trade good. Due to the prevalence of its historic use, we hypothesized that *C. lanceolata* corms would be rich in dietary mineral nutrition, and compare favorably to cultivated food plants. We also expected differences in the average dietary mineral content of corms from different populations, due to the heterogeneity of the environments where *C. lanceolata* grows. Lastly, we expected differences in mean dietary mineral content of corms across their phenology due to use/storage of dietary minerals over the course of the flowering cycle. To test these predictions, we sampled 12 populations across the Columbia Plateau region of North America. We resampled six populations about 30 days after our initial harvest. Corms were assayed for dietary mineral and toxic metal content using standard ICP-OES methods. We found that a single serving of corms (100 g fresh weight) likely provided between 10-25% of the DRI for Mg, P, Cu, and Zn, and over 100% of the DRI for Fe and Mn. These values compare

favorably to modern foods. The average content of macroelements did not vary significantly with mode of preparation (i.e. removal of the periderm), yet concentrations of trace elements Fe, Cr, Cu, Mn, and Pb in the samples was significantly higher ( $p < .001$  for Fe, Cr, Pb, Al;  $p = .003$  Cu;  $p = .007$  Mn) between samples with periderm intact and samples with the periderm removed. The average amount of Pb in the samples was significantly reduced ( $p < .001$ ) when the periderm was removed prior to analysis. Of the six populations resampled, the average amount of most macroelements and one trace element contained in the corms increased significantly (Na;  $p = .05$ , Ca;  $p = .004$ , Mg;  $p = .015$ , K;  $p = .006$ , Cu;  $p = .043$ ) in the late samples. Concentrations of toxic metals did not change significantly between sample times. The mean weight of corms was significantly different among populations ( $p < .001$ ). Averages concentration of macroelements (Ca;  $p = .04$ , Mg;  $p < .001$ , K;  $p = .004$ , Na;  $p = .004$ , P;  $p = .013$ ), trace elements (Fe;  $p = .004$ , Mn;  $p = .023$ , Zn;  $p < .001$ ) and toxic metals (Cd, Pb, Ba, Al;  $p < .001$ ) differed significantly by sample location. These results suggest that corms are a viable but highly variable source of nutrition. Consumption of corms can increase health by displacing less nutritious modern foods, and could increase micronutrient consumption. Corms may mitigate the risk of common deficiencies, especially when preparation methods, time of harvest, and place of harvest are considered. Our results call into question the validity of previous studies with low replicate samples.

## **Acknowledgements**

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## **Master's Thesis**

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## **Introduction**

### **History and Importance of Food Plants**

Undoubtedly food plants have an essential role in meeting the nutritional requirements of humans. Our genus (*Homo*) appeared between 2.4 - 2.8 million years ago (mya), and the hunting and gathering (foraging) way of life sustained our species for most of its existence. Comprehensive nutritional studies on the diet of many foraging societies were not conducted prior to westernization, yet reconstructing the traditional diet of foragers is possible using modern nutritional information combined with ethnographies and the archaeological record (Eaton and Konner 1985, Eaton et al. 1997). For example, dietary reconstruction studies from Lee's (1968) study compiling information from 862 societies, initially assumed a plant to animal food ratio of 65:35. More recently, studies estimate that about 75% of the evaluated foraging societies derive between 35-44% of their sustenance directly from plant foods (Cordain et al. 2000). A shift from foraging to food production occurred during the Neolithic Revolution, and modern studies estimate that about 90% of caloric intake worldwide now comes from 15 species, and four of those species (rice, maize, sorghum and wheat) contribute about 50% of calories consumed worldwide (FAO 2009).

### **Evidence for the Decline of Health Associated with the Neolithic Revolution**

The shift from a foraging lifestyle to food production occurred at the beginning of the Holocene, when people in seven regions (Eastern Mediterranean, China, New Guinea, Ethiopia, Eastern North America, Mesoamerica and South America) began practicing agriculture as a means of food production (Bellwood 2005). The worldwide shift from foraging to agriculture, known as the Neolithic Revolution (Childe 1936), coincides with a dramatic increase in artifact remains, which are interpreted as the beginning of a continuous period of population growth (Bocquet-Appel 2011). This shift dramatically changed diets and population density worldwide. Decreased diversity and increased dependence on marginally nutritious food sources cause physiological changes in humans, which are well documented in the archaeological record following the shift to an

agricultural lifestyle (Larsen 1995). In addition to increased population density, evidence associated with this dietary shift includes decreasing height, age span, and declining oral health.

In the course of a few centuries after the shift to food production, human population densities increased from one forager to 20 or more agriculturalists per square mile (Johnson and Earle 2000). The growth trend continues, resulting in a thousand-fold increase in global population from around six million people at the advent of agriculture (Biraden 1979) to the current estimate of 7.2 billion. The population explosion is attributed to two major factors. First, having children is less costly for agriculturists, and second food production per unit of land increased (Price and Gebauer 1995). Having and caring for children interfered with the nomadic lifestyle of many foraging societies (Locay 1983). Children in foraging societies contribute little or nothing to family subsistence (Kent 1996). In contrast, the children of agriculturalists contributed substantially more to food production (Kramer and Boone 2002), and agrarian children often began working at an early age (Ulijaszeka 1993). The more reliable and readily available food source from agricultural products increased maternal fertility by reducing the birth interval (Valeggia and Ellsion 2004). The lower cost of having children coupled with increased maternal fecundity increased the human population exponentially.

The agricultural lifestyle reduced diversity in the diet, increased intake of less nutritious foods, and encouraged a more sedentary lifestyle. People began to get most of their nutrient intake from relatively few plant cultivars (Grivetti and Ogle 2000). The diverse group of edible wild species that foragers once consumed were reduced, and eventually eliminated from the diet (Grivetti 1981). Currently, humans get ~90% of our caloric intake from 15 species: eight cereals (wheat, rice, corn, barely, millet, sugar cane, sorghum, and rye) and four tubers (cassava, potato, sweet potato, and yam). Archeological evidence and modern day observations of societies still practicing low-technology agriculture show that their diets are dominated by one or a few marginally nutritious plants, e.g. maize in the Americas, rice in Asia, wheat in Europe, and millet or sorghum in Africa (Larsen 1995). Maize is lacking a number of essential amino acids, and whereas it contains the essential vitamin niacin, it is chemically bound and thus unavailable for absorption. Moreover, iron absorption from maize is poor, thus iron

deficiencies are common in maize dominated diets (Ashworth et al. 1973, FAO 1990). Likewise, rice can inhibit vitamin A uptake, even when vitamin A is procured from other sources (Wolf 1980), and millet and wheat are already low in iron, and processing further lowers iron content (Carlson et al. 1974 from Larsen 1995).

Changes in human physiology and anatomy, such as dental health, development, and age at death are documented in the archaeological record after the Neolithic revolution, and these differences are attributed to the changes in food composition, in both dietary diversity and cooking methods. Because of the diverse, nutrient dense characteristics of a foraged diet, foragers would have met or exceeded currently recommended vitamin and dietary mineral allowances either absolutely or relative to energy intake (Eaton et al. 1996). The foraged foods of pre-agricultural humans contained higher levels of micronutrients (vitamins and dietary minerals) relative to energy than most commonly consumed agricultural products, especially considering how many agricultural products are prepared for consumption (Eaton and Konner 1985). Tooth loss is caused by a variety of factors, but many studies suggest that the increase in cavities corresponds with an increase in tooth loss, suggesting a relationship between these two conditions (Larsen 1995). The decline in oral health is largely attributed to an increase of lower fiber foods, carbohydrate rich foods, as well as methods of preparation. High fiber foods mechanically cleanse teeth, whereas carbohydrates are linked to increased cavity prevalence and tooth loss when organic acids demineralize dental hard tissue during the process of bacterial fermentation of dietary carbohydrates (Newbrun 1982). Many agriculturalists prepare plant food by boiling them into a soft consistency, which can promote the growth of bacterial colonies in areas of the mouth, which are not mechanically cleansed by consumption of high fiber foods (Larsen 1995). Since oral health is correlated with overall general health good oral health reduces premature mortality, and is often a determinant factor for overall quality of life (WHO 2003). Studies show an increase in the percentage of teeth with cavities after the adoption of an agricultural lifestyle (Larsen 1995). For example, Turner's (1979) study showed that the average frequency of teeth affected by cavities in foragers was 1.7%, compared to 8.6% of agriculturalists.

Developmental differences such as decreased growth rates, shorter overall stature and younger age at death are documented in the skeletal remains of humans after the Neolithic Revolution, and are generally attributed to an increased dependency on low or poor nutritional quality food (Eveleth and Tanner 1990). Growth rates appear to be a reliable indicator of overall nutritional status (Gracey 1987) and people who are short for their age are generally unhealthier than individuals that are tall for their age (Cook 1984). Children in populations with unmet nutritional requirements are often shorter in stature than their counterparts with sufficient nutrition in their diets (Huss et al. 1985, Bogin 1988, Eveleth and Tanner 1990), and analyses of bones from prehistoric North America shows that children of agricultural societies are shorter in stature for their age group than their foraging counterparts (Sauders 1992). Adult height is determined by several factors, but evidence from studies reveals a strong relationship between growth impediment at a young age and terminal body size (Larsen 1995). Many studies show a decline in the average height of societies after their adoption of agricultural lifestyle (Perzigian et al. 1984, Nickens 1976, Meiklejohn et al. 1984, Larsen 1984, Larsen 1982, Kennedy 1984, Goodman et al. 1984). Lastly, the skeletal remains of humans from agricultural societies suggest they were younger at death than their foraging counterparts, and this provides evidence for declining life expectancy after a shift to an agricultural lifestyle (Kobayashi 1967, Welinder 1979, Goodman et al. 1984, Kennedy 1984, Larsen 1987).

### **Back to the Future: Understanding our Evolutionary Past**

Evolutionary theory suggests when the ecosystem stays relatively constant stabilizing selection should maintain a genotype that is best suited for the environment. However, when the environment begins to change, individuals can experience discord between their genome and the environment, and directional selection should shift genotypes towards conditions best suited to the new environment. Over several million years, the genetic traits of our genus were optimized for a foraging lifestyle, and after the Neolithic Revolution, the dramatic shift in diet may have produced a discord between environment and genome. Modern people are living in an environment that would be unrecognizable to our ancestors. The change from foraging to food production may have occurred too recently on the evolutionary timescale for the human genome to adapt

(Eaton and Konner 1985, Eaton and Konner 1988, Nesse 1994, Boaz 2002, Cordain et al. 2005), which studies suggest can manifest as disease (Nesse 1994), and can cause or exacerbate “the diseases of civilization” (Eaton and Konner 1985, Eaton and Konner 1988, Nesse 1994, Cordain et al. 2005). Diseases of civilization include coronary heart disease, obesity, hypertension, type two diabetes, cancers, autoimmune disorders, and osteoporosis, which were rare or absent in pre-agricultural (and non-westernized) societies (Carrera-Basto et al. 2011). These chronic degenerative diseases are the leading causes of death in modern people (CDC 2010), and disproportionately high in subsets of the population that have more recently experienced the transition from foraging to agriculture, such as Native American groups.

When compared to the general population, by nearly every indicator, the overall health of Native Americans is poor (Welty 1991, Young 1994, Indian Health Service 1996, Amparo et al. 2011, Cobb et al. 2014, Espey et al. 2014). Levels of obesity are high among all groups but are increasing alarmingly fast in young people (Welty 1991, Story et al. 1999). Historically, diabetes was absent in Native American populations, yet current estimates list diabetes is a major cause of morbidity and mortality in people of Native American descent (Welty 1991, Gohdes 1995, Espey et al. 2014). Likewise, cardiovascular disease, which was rarely noted in the earlier part of the 1900s, is now the leading cause of death in Native Americans (Welty 1991, Espey 2014). In 2003, the U.S. Commission on Civil Rights published *A Quiet Crisis: Federal Funding and Unmet Needs in Indian Country*, which detailed the disparity in overall health between Native Americans and the U.S. general population. On average, Native Americans have a lower life expectancy by six years compared to other racial groups and the rate of mortality of those under 25 is three times the national average. When compared to their non-native counterparts, Native Americans are 12.5% more likely to be obese (Cobb et al. 2014), are eight times more likely to have diabetes (Lang 2006), and almost two times more likely to die from cardiovascular disease (Howard et al. 1999). Fortunately, Espey et al. (2014) concluded that much of the observed morbidity and mortality could be addressed through known risk mitigation strategies such as increasing the consumption of micronutrients, exercise, and changes in diet.



In contrast to the epidemic levels of disease in modern cultures, the lack of disease in pre-agricultural societies, a lifestyle and diet that mimics our foraging ancestors could be an effective risk mitigation strategy (Brock and Diggs 2013). To combat health problems associated with modern lifestyles, many initiatives espouse the benefits of foraging. Finding, harvesting, and preparing foraged foods generally leads to increased nutrient intake, displacement of less nutritious foods, and an increase in physical activity, all of which can benefit overall health. In addition, comparative studies indicate that wild food plants are often more nutrient dense than their cultivated counterparts (Nelson et al. 2000, Booth et al. 1992, Sakai 1983, Coursey 1983, Widdowson 1992, Wardlaw and Insel 1995), and increasing micronutrient intake has been hypothesized as one of the most cost effective public health interventions available (Jamison et al., 1993; Tulchinsky, 2010; Harrison, 2010). Micronutrient deficiencies are among the top 20 risk factors for impaired quality of life (Egeland and Harrison 2013). Additionally, micronutrient deficiencies among indigenous groups tend to be at higher levels than that of the general population, thus foraging could improve their overall health. National initiatives, such as the Center for Disease Control's Traditional Foods Project provides funding to educate groups on how to properly identify, harvest, process and cook traditional foods. Many tribal groups have their own traditional food programs, and in a report by the Traditional Foods of Puget Sound Project, there are at least 14 programs in the Puget Sound Area of Washington State alone that offer training in foraging and preparing traditional foods (Krohn 2010). More regionally, initiatives such as the Kalispel Educators Encampment, Colville Confederated Tribes Diabetes Program, and the Northwest Indian College's Traditional Plants and Foods Project encourage people to forage foods as a way to mitigate the risk of modern disease.

Forming nutritional plans that include foraged foods is difficult without comprehensive nutritional information. Access to comprehensive nutritional information and an understanding about how nutritive content can differ spatially and temporally in foraged foods could benefit foragers and may support increased consumption of traditional foods (Phillips et al. 2014). Unfortunately, little is known about the nutritional quality of many foraged plant foods (Kuhnlein and Turner 1991, Kuhnlein 2000, Grivetti and Ogle 2000), and nutritional information is difficult to locate and assemble, especially

without formal training (Grivetti et al. 1987, Grivetti and Ogle 2000). Where nutritional information on foraged plants is available, the data are usually regional and based on incomplete studies. However, the growing attention on foraging has led to recent studies that use modern techniques to assess the nutrient values of some wild food plants (Phillips et al. 2014). Typically, research on foraged foods are from a single (or few) samples at a single point in time, which may not be representative of the actual nutritional value. For example, the nutritive content of some agricultural products differs due to time of harvest (phenology) (Remorini et al. 2008, Venneria et al. 2012) and environmental conditions (population). Potential differences between populations or phenology are generally not examined in wild food plants (Grivetti and Ogle 2000). To further complicate matters, inconsistent project designs and methods makes comparison difficult at best (Kuhnlein and Turner 1991, Kuhnlein 2000, Grivetti and Ogle 2000). As a case in point, the available nutritional information on *Claytonia lanceolata* is based on a single incomplete study. A proximate analysis of *C. lanceolata* corms was performed in 1938 and to date this single analysis has served as the sole source of nutritional information for the species (Yanovsky and Kingsbury 1938). Moreover, Yanovsky and Kingsbury's (1938) test does not address the taxonomy of the species (i.e., no deposited voucher specimen), so independent confirmation of species identification is not possible and data on the population where the corms were procured is not published, thus, their tests appear to be from a single population at a single point in time.

Traditionally, indigenous peoples harvested the underground stems (corms) of *C. lanceolata*, a subalpine to montane herbaceous perennial in the family Montiaceae to eat immediately, trade, or store for later consumption (Teit 1928, Verne 1932, Turner et al. 1980, Mastrogiuseppe 2000, Palmer et al. 2000, Ross 2011). *Claytonia lanceolata* was likely one of the first available food plants in the spring, due to the early flowering cycle (i.e., April and May) and grows in a number of different environments. In modern times, *C. lanceolata* is often overlooked as a food plant, yet early records list the corms as an important food source (Teit 1900, Teit 1928, Verne 1932). For example, Teit's ethnography (1928) about the Salishian tribes of the Western Plateau, *Claytonia sessifolia* (a synonymous scientific name for *C. lanceolata*) is listed as one of the most important food plants to the Thompson and Okanagan People. Some plateau groups included *C.*

*lanceolata* in their religious ceremonies (Teit 1928, Verne 1932, Turner et al. 1980, Ross 2011), and even incorporated *C. lanceolata* into their cultural explanation of the stars (Boas 1921). In addition, bereaved women in some groups were told to pull up and spread the dried stems to areas they didn't grow as a way to ease their suffering (Turner 2015).

## **Purpose and Hypothesis**

To better understand how the nutritive content of *C. lanceolata* corms may have influenced the health of native peoples in this area, I assayed the mineral content of *C. lanceolata* corms. I analyzed the dietary mineral and toxic metal content of corms from multiple populations, compared samples that had the periderm removed prior to analysis, and measured dietary mineral and toxic metal concentrations from a subset of the same populations at different times (phenology). Due to its historical use, I predicted *C. lanceolata* corms would be a good source of dietary minerals for regional native peoples (Table 1; Appendix 1), yet hypothesized mineral nutrition would vary due to mode of preparation (i.e., removal of the periderm versus periderm intact), time of harvest (early versus late) and site location. Because we sampled at sites with known toxic metal contamination (i.e. the Coeur d'Alene basin), toxic metals detrimental to human health were also assayed (Table 2; Appendix 2).

## **Methods**

### **Site Location and Sample Collection Protocol**

The 12 selected sample sites encompass a range of environments where *C. lanceolata* grows (Map 1). Sampled populations were identified by word of mouth (Badger Mountain, St. Paul's Mission, Huetter Rest Area, Kamiak Butte), by herbarium

specimen record (Leader Lake, Sherman Creek, Mud Lake, Tubbs Hill, Robinson Park, Moscow Mountain) and by searching without prior knowledge (Leader Lake, Elk, Chattaroy). I collected *C. lanceolata* corms from April and May of 2014 (Table 3), and six populations (Mud Lake, Huetter Rest Area, Chattaroy, Elk, and Tubbs Hill) were resampled approximately 30 days after their initial sampling date (Map 2).

GPS coordinates (WGS-84) for each site were recorded using a handheld GPS unit (Magellan eXplorist 310). Individuals were sampled within 50 m of the initial coordinate reading. Corms from every other noticed individual, irrespective of plant size, were dug using a slender pry bar to minimize disturbance to the site. About 50 g of fresh corms were collected from each site, and whole individuals were placed in a gallon storage bags (Ziploc) in a cooler, and returned to the lab for processing.

### **Sample Processing**

Individuals were examined and a representative series of photos were taken to characterize diversity at each site. Voucher specimens for each population and at each sample time were prepared and are stored in the Eastern Washington University Herbarium.

Corms were removed from their above ground biomass, triple rinsed by hand in de-ionized water and air dried prior to weighing. Weighed corms were randomly assigned to peeled or unpeeled treatment groups. Corms in the peeled treatment had the periderms manually removed with a razor blade. Peeled and unpeeled corms were oven dried at 57°C for seven days after which they were randomly divided into 1.5 g groups, hand homogenized using a mortar and pestle, and stored in 5 ml polyethylene tubes (Fisher Scientific) at room temperature prior to subsequent analysis.

### **Dietary Mineral Analysis**

*Claytonia lanceolata* dietary mineral content was determined by Inductively Coupled Optical Emission Spectrometry (ICP-OES, Thermo iCap 6200) under the supervision of Dr. Carmen Nezat, in the Eastern Washington University Environmental

and Analytical Geochemistry Laboratory. Approximately 1 g of homogenized tissue per sample was transferred to pre-weighed 150 ml beakers, and digested according to EPA Method 3050B. Prior to use, all lab wear was soaked in 5% HNO<sub>3</sub> for 24 hours and thoroughly rinsed with deionized water. In the fume hood, 10 ml of 1:1 HNO<sub>3</sub> to H<sub>2</sub>O was added to each beaker, mixed by swirling, and covered with a watch glass. Each beaker was placed on a hot plate and the sample heated to approximately 95°C, then refluxed (cooled without evaporative loss) for 15 minutes then removed from the hot plate to cool to room temperature. After cooling, 5 ml of concentrated nitric acid (67-70%, OmniTrace) was added to each sample, and heated to approximately 95°C. Each time brown fumes were observed, an additional 5 ml of concentrated HNO<sub>3</sub> was added until no further reaction occurred. Samples were then reduced until total volume was approximately 5 ml, and then 2 ml of H<sub>2</sub>O, and 3 ml of H<sub>2</sub>O<sub>2</sub> (35% H<sub>2</sub>O<sub>2</sub>, Hydrogen peroxide for Analysis by Acros Organics) were added to each beaker. If bubbles formed rapidly, additional H<sub>2</sub>O<sub>2</sub> was added in 1 ml increments (not exceeding 10 ml) until no reaction was observed. Samples were then reduced to approximately 5 ml, allowed to cool, and filtered through Whatman 41 (GE) filter papers in funnels into 50 ml volumetric flasks. Beakers and funnels were flushed with nanopure water until the solution was diluted to 50 mL. The solution was then placed in 50 ml polyethylene vials (Fisherbrand) and stored at room temperature overnight.

Reported dietary mineral concentrations represent the total recoverable portions from each sample (Table 5). Reference standards for soil and orchard leaves (CRM-Soil B and CRM-OL: High Purity Standards) were analyzed to monitor the accuracy of dietary mineral analysis. Further quality control measures were taken by regularly analyzing procedural and reagent blanks during the course of analysis. The following elements were quantified: Aluminum (Al), Barium (Ba), Calcium (Ca), Cadmium (Cd), Chromium (Cr), Copper (Cu), Iron (Fe), Potassium (K), Magnesium (Mg), Manganese (Mn), Sodium (Na), Phosphorus (P), Lead (Pb), and Zinc (Zn). Only those dietary minerals and toxic metals relevant to human health were further analyzed. The average dietary mineral composition of corns will be entered into the USDA Nutrient Database for Standard Reference, release 20 (USDA, 2007) in a distinct food group, I.e. American Indian/Alaska Native foods (USDA, 2011).

## **Serving Size Estimation**

Serving size seems to be a mostly arbitrary construct, so using a 100 g fresh weight allowed for ease of comparison of other reported dietary mineral values in other foods. A serving size is defined by the FDA as the amount of food customarily consumed per eating occasion (FDA 2015). The exact formulas used to determine serving size are not published, but demographic and socioeconomic characteristics, as well as the age class of the relevant population are considered. Potatoes and yams are generally considered to have a 110g fresh weight serving size and the serving size for many other vegetables is 85g fresh weight. For the purpose of this study, we assumed a single serving to be 100 g fresh weight, close to the average between a potato and vegetable (97.5g).

## **Statistical Analyses**

To determine if subsets of corms from different populations, subsets with periderm removed, and subsets from early and late sample times contained statistically significant differences in the average concentration of the dietary mineral and toxic metals, we compared the averages using a series of Kruskal-Wallis tests, due to nonparametric data. Except as noted, statistical tests were performed using Systat 13 and SigmaPlot 11.0 (Systat Software, Inc.). The significance level for all analyses was ( $\alpha \leq 0.05$ ). The average macroelement, trace element and toxic metal concentration was reported using the mean value for all unpeeled samples (n=72). To determine if preparation (i.e., periderm removal) affected the concentration of dietary minerals and toxic metals, we pooled subsets with periderm removed (n=18) and periderm intact (n=54). Early and late harvest was compared by pooling unpeeled sample subsets from both harvest times (n=18), and then to determine which populations varied from early to late sample time, we compared subsets (n=3) from each specific population sampled multiple times using a series of paired T-tests (e.g. Mud Lake early versus late).

## Results

Corms from 12 populations, 72 samples total, contained elements essential to human health (Ca, Mg, K, Na, P, Cu, Fe, Mn, Cr, Zn; Tables 2 and 3), and the fresh weights of the individual corms varied from 0.006 g to 11.546 g (mean weight 0.811 g;  $n=1270$ ; Figure 1). On average, a single serving of corms provides >10% of the DRI of the macroelements Mg and P, >10% of all trace elements (Cu, Fe, Mn, Cr, Zn), and are generally low (<.01 mg/100g) in heavy metals such as Cd, Pb, and Ba. When compared to cultivated foods with similar morphologies, such as potatoes and turnips, corms have higher values for the macroelements Mg, and P, and most trace elements (Fe, Mn, Cr, Zn) (Figure 5).

The effect of the mode of preparation (i.e., removal of the periderm or periderm intact) did not alter the average content of macroelements, yet concentrations of trace elements Fe, Cr, Cu, Mn in unpeeled samples was significantly higher ( $p<.001$  for Fe, Cr, Pb;  $p=.003$  Cu;  $p=.007$  Mn). The average concentration of Ba and Cd was not significantly different in peeled versus unpeeled samples, however the average amount of Pb was significantly reduced ( $p<.001$ ) when the periderm was removed prior to analysis. Lead over the European Union Maximum Limit (EURL; Pb >.10 mg/100 g) was found in three populations: Huetter Rest Area, Sherman Creek, and St. Paul's Mission. The Mud Lake and Tubbs Hill populations were also close to the EURL (Table 2), and Cd levels that surpassed the EURL (>.15 mg/100 g) were found in two populations: Sherman Creek and St. Paul's Mission.

Averages concentrations for most micronutrients increased (Na;  $p=.05$ , Ca;  $p=.004$  Mg;  $p=.015$ , K;  $p=.006$ ) and one trace element (Cu;  $p=.043$ ) increased over the phenology. Concentrations of toxic metals did not change significantly between sample times.

All sites varied in their dietary mineral concentrations, however there were no populations with uniformly high or low values. The mean weight of corms was significantly different among populations ( $p<.001$ ), as was the average content of

macroelements (Ca;  $p=.04$ , Mg;  $p<.001$ , K;  $p=.004$ , Na;  $p=.004$ , P;  $p=.013$ ), trace elements (Fe;  $p=.004$ , Mn;  $p=.023$ , Zn;  $p<.001$ ) and toxic metals (Cd, Pb, Ba;  $p<.001$ ). More specifically, corms assayed had a wide range of variation for macroelements:

The average Ca was 22.1 mg /100 g, and ranged from 14.5 – 46.8 mg/100 g; the average Mg concentration was 38.4 mg /100 g and ranged from 21.6 -89.0 mg /100g; the average K concentration was 250.1 mg / 100 g, and ranged from 171.2 to 475.9; the average Na concentration was 3.2 mg / 100 g, and ranged from 1.8 to 7.7 mg / 100 g; the average P concentration was 95.6 mg / 100 g, and ranged from 51.7 – 253.8 mg / 100 g. Trace elements concentrations for corms was also highly variable: the average Cu concentration was 0.12 mg / 100 g, and ranged from .08 - .31 mg / 100 g; the average Fe concentration was 23.1 mg / 100 g, and ranged from 4.0 – 120.9 mg / 100 g; the average Mn concentration was 2.1 mg / 100 g, and ranged from 0.7 – 5.1 mg / 100 g; the average Cr concentration was 0.03 mg / 100 g, and ranged from 0.01 – 0.18 mg / 100 g; the average Zn concentration was 95.6 mg / 100 g, and ranged from 51.7 – 253.8 mg / 100 g. The toxic metal concentration of corms assayed was variable: the average Cd concentration was 0.05 mg / 100 g, and ranged from 0.01-0.36 mg / 100 g; the average Pb concentration was 0.10 mg / 100 g, and ranged from 0.01 – 0.49 mg / 100 g; the average Ba concentration was 1.0 mg / 100 g, and ranged from 0.2 – 2.1 mg / 100 g.

## Discussion

Our results corroborate what generations of Native peoples likely understood. The wild foraged food, *C. lanceolata* is an excellent but highly variable source of dietary mineral nutrition. *Claytonia lanceolata* corms contain all analyzed macroelements and trace elements, and are an excellent source of some elements people are often deficient in (Mg, Fe, Zn), but harvest location matters.

This study assessed the dietary mineral composition of *C. lanceolata* corms from the Columbia Plateau region of the Pacific Northwest to determine the overall dietary



mineral content, and to determine if dietary mineral concentrations varied by method of preparation (peeled vs. unpeeled), time of harvest (early vs. late), or by harvest location. Overall, our results suggest that inclusion or reintroduction of *C. lanceolata* corms into the diet may be useful in mitigating health problems by increasing dietary mineral nutrition intake and displacing less nutritious foods. These findings add to a growing body of knowledge that suggests that wild food plants can be nutritionally superior to modern cultivars.

### **Dietary Mineral Nutrition**

*Claytonia lanceolata* corms are a good source of Mg (38mg/100g) and surpass many modern foods considered high in Mg, such as avocados (29mg/100g), and bananas (27mg/100g). Whereas most people get adequate levels of P in their diets, some conditions such as diabetes, alcoholism, and starvation can cause levels of phosphorus in the body to decline. On average, a serving of *C. lanceolata* corms provides a portion of all macroelements, and > 10% of the DRI of the macroelements Mg and P. Magnesium deficiency (hypomagnesemia) is common and affects between 2.5 -15% of the general population and only about 32% of the U.S. population meets the RDI for magnesium (Ayuk 2014).

Trace elements in *C. lanceolata* corms were also found at high levels (Table 2), and on average a single serving of corms would provide > 100% of the daily values of Fe, Mn, and Cr, and between 10-25% of Cu and Zn. Deficiencies in Fe and Zn are relatively common. The World Health Organization (WHO) estimates that 3.7 billion people are iron deficient with two billion considered anemic (WHO 2011), and in the US alone, around nine million people are clinically deficient with about 7% of the population ingesting less than 50% of the RDI (Wilson et al. 1997). Zinc deficiency is likewise problematic in some developing countries, with low zinc intake contributing to pregnancy complications, decreased disease resistance, impaired growth, and genetic disorders (Allen et al. 2006). Deficiencies in other trace elements such as Cu, Mn, and Cr are rare, but problematic to human health. Copper deficiency can be caused by increased consumption of Zn used in the prevention or treatment of common colds, ulcers, celiac disease, acne and other ailments. The daily requirement of Mn is low (2mg/day), thus

deficiencies are unlikely and Cr deficiency has only been observed in hospital patients who were intravenously fed for long periods of time (Review of Chromium 2002).

Although our results suggest a single serving of *C. lanceolata* corms could provide adults with adequate amount (or more) of iron on a daily basis, we make note of the fact that the Fe in *C. lanceolata* corms is not the heme iron found in meats, which generally has higher bioavailability and is less affected by other dietary components that affect iron uptake, such as vitamin C (which increases uptake of nonheme iron).

## **Toxicity**

Our regional proximity to known contamination sites (i.e., Coeur d'Alene basin) made us sensitive to the potential for exposure to toxic metal contaminants by modern foragers. To this end, we analyzed corms for toxic metal content with and without the periderm where adherence of soils particles increases the likelihood of toxic metal ingestion. Concentrations of the toxic metals we tested for (Cd, Pb, and Ba) were generally low, with the exception of the Huetter Rest Area, Sherman Creek, and St. Paul's Mission sites where we saw elevated levels of Cd and Pb.

We hypothesize the proximity of these sites to potential contamination sources (e.g. highways, major water bodies, previous mining locations, and agricultural areas) is one possible explanation for the observed elevated concentrations. Sherman Creek and St. Paul's Mission are proximate to the Columbia River and a major highway (US-2), both of which provide a potential means for contamination from Cd and Pb. High levels of Cd and Pb at the Huetter Rest Area site are likely explained by its proximity to a major Interstate (I-90). Cadmium and Pb are both contaminants from fossil fuel combustion. Additional analyses examining the relationship between proximity to major highways and elevated Cd and Pb levels in wild food plants are warranted.

Although the effects of barium on human health are not well understood, the EPA has determined that drinking water should contain no more than 2.0 mg of barium per liter of water. To determine the safe levels of Ba in food, we used a 4 mg/per serving limit extrapolated from the EPA safe levels in drinking water limits. All populations tested contained quantities much lower than this threshold.

## **When, Where and Whether to Peel or Not to Peel**

The average concentration of various dietary minerals and toxic metals significantly changed with exclusion of the periderm, phenology and sample site location. We suggest that soil adhesion to the periderm may be a critical contributor to these differences. Our results suggest that removal of the periderm decreases the average dietary mineral content of trace elements and Pb in corms. At sites with a low likelihood of toxic metal contamination, leaving the periderm intact can increase dietary mineral intake.

Harvesting later in the above ground growth cycle (phenology) increases the concentration of most macroelements, but has no effect on trace element concentrations. This result is consistent with our hypothesis and can be explained by the storage of nutritive content in the corms over the course of the above ground growth cycle. As spring ephemerals, *C. lanceolata* preform their flowers for the next year and use stored nutrients to resume growth early in the following year, prior to the expansion of their photosynthetic leaves. Dietary mineral content appears to increase incrementally over the course of the above ground growth cycle (Fig. 9 and 10), suggesting that mineral content stored in the corm is allocated to vegetative growth early in the season and that storage of mineral content begins again after photosynthetic leaves are expanded. The single exception is P, which is required in large amounts for vegetative growth. Additional studies might examine if the P content of soils influences the distribution and growth characteristics of *C. lanceolata*.

At the population level, we began to notice site-specific dietary mineral concentrations (Table 2, Maps 2, 3, 4). One possible explanation for this wide range of dietary mineral concentration is variation in soil types. The Soil Survey Geographic Database (SSURGO) data, overlain with reference points of known *C. lanceolata* populations provides evidence for of the wide range of soil types in which *C. lanceolata* grows. For example, in one four mile area around the St. Paul's Mission site, at least 20 specific soil types exist, and *C. lanceolata* grows in at least six of these soils. This pattern of high soil type diversity over relatively small spatial scales is consistent across all

sample sites (Map 6), and may explain the highly variable, yet inconsistent pattern of dietary mineral nutrient content we see over our sample sites.

It is likely that indigenous peoples with dietary mineral deficiencies, through a process of trial and error, could have discovered specific harvest areas with increased levels of dietary mineral nutrition that could alleviate deficiency symptoms. Symptoms of dietary mineral deficiencies are wide ranging, but often come in the form of malaise, and after ingestion of sufficient dietary mineral nutrition, alleviation of the symptoms occurs rapidly. Iron deficiency during the winter months when food scarcity was highest would have manifested as weakness, fatigue, headaches, shortness of breath, and difficulty concentrating (Table 1), which would have been quickly alleviated by ingesting *C. lanceolata* corms from populations with high Fe content. Sites with high levels of Fe in the soil and/or corm would have provided an important nutritional boost to women, juveniles, and the elderly in particular. Our current understanding of iron deficiency especially during pregnancy suggests it can cause low birth weight, premature birth, and impaired cognitive and behavioral ability, as well as adverse and often irreversible cognitive and psychological affects in infants (Aggett 2012, Baker and Greer 2010). Insufficient iron in the elderly, especially those with other health conditions (cancer, celiac disease, heart disease) is associated with increased risk of hospitalization and mortality (Riva et al. 2009). In our study, iron content of corms from specific sample sites ranged from 4 to 121 mg per serving. For comparison, modern iron supplements come in ranges of 35 to 100 mg per pill. Harvesting from specific areas with high levels of iron in the soil would have been as effective as modern supplements for curing iron deficiency.

### **Protecting Indigenous Knowledge**

Traditional or cultural knowledge about harvest areas and preparation methods of plant foods is disappearing at an alarming rate (Inglis 1993, Heyes and Jacobs 2008, Garcia et. al 2013). Information about places of harvest that have higher nutritive content would likely be preserved in cultural knowledge. There is some evidence of this today in the maintenance of family or community harvest areas (Krohn 2007). Hunn (1981) estimated that people of the plateau culture area got up to 70% of their calories from plant foods, and about 50% of those calories from root foods, thus protection of indigenous

harvest areas are as equally important to preserving indigenous tradition and knowledge as other cultural concerns, such as protection of salmon runs or language preservation. Our results suggest greater consideration for the protection and preservation of historical harvest areas is warranted if we aim to protect and preserve traditional knowledge of the plateau culture area.

### **Modern Nutritional Testing of Wild Plant Foods**

Reported values of wild food plant nutritive content is often based on studies with low replicate samples, and our results suggest that these studies are incomplete. Studies with low replicate samples can significantly over or under report average dietary mineral concentrations. Using these reported values to inform management decisions, or in downstream publications could misrepresent the factual importance of a particular species. If the nutritional content of other food plants is similar to *C. lanceolata*, studies should consider sampling more broadly across a species distribution and where relevant across the phenology. Unfortunately, some studies suggest nutritional testing of wild food plants is limited by funding, availability of sample material, and time (Kuhnlein and Turner 1991, Kuhnlein 2000), however in light of our results; we question the validity of these studies with limited sample replicates.

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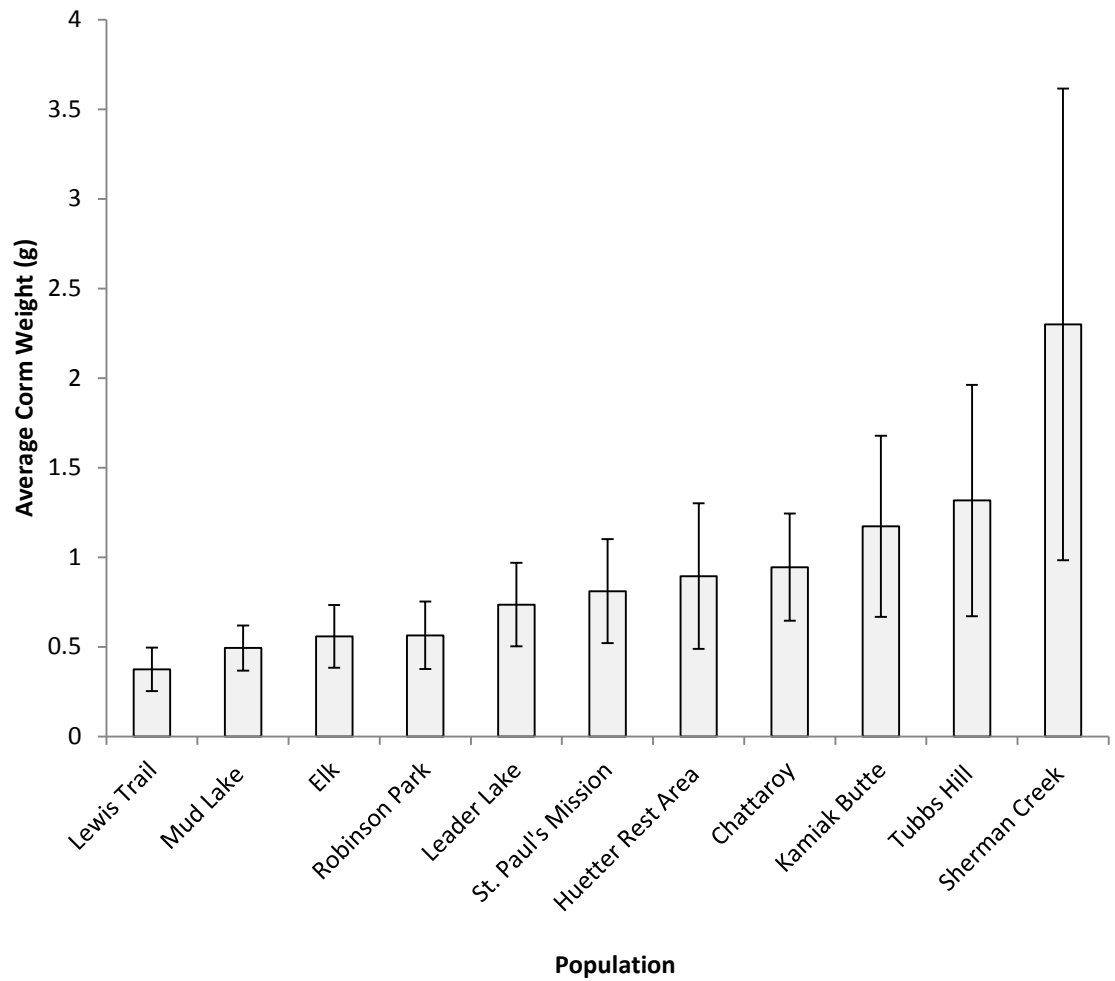


Figure 1. Mean weight of *C. lanceolata* corms from select populations (n=11). Error bars represent +/- 1 standard deviation.

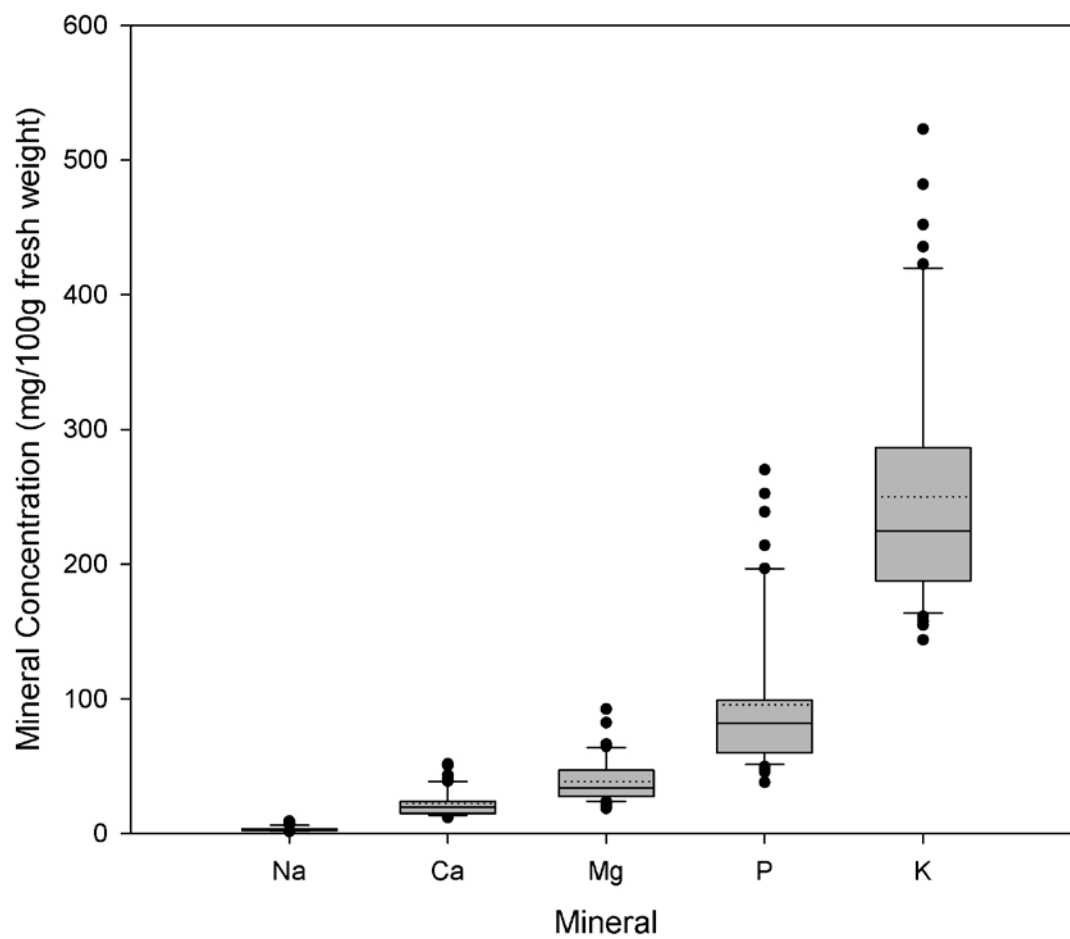


Figure 2. Macroelement content of *C. lanceolata* corms from all populations (n=72). Solid lines represent the median values; dotted lines represent the mean values; outliers are represented as solid circles.



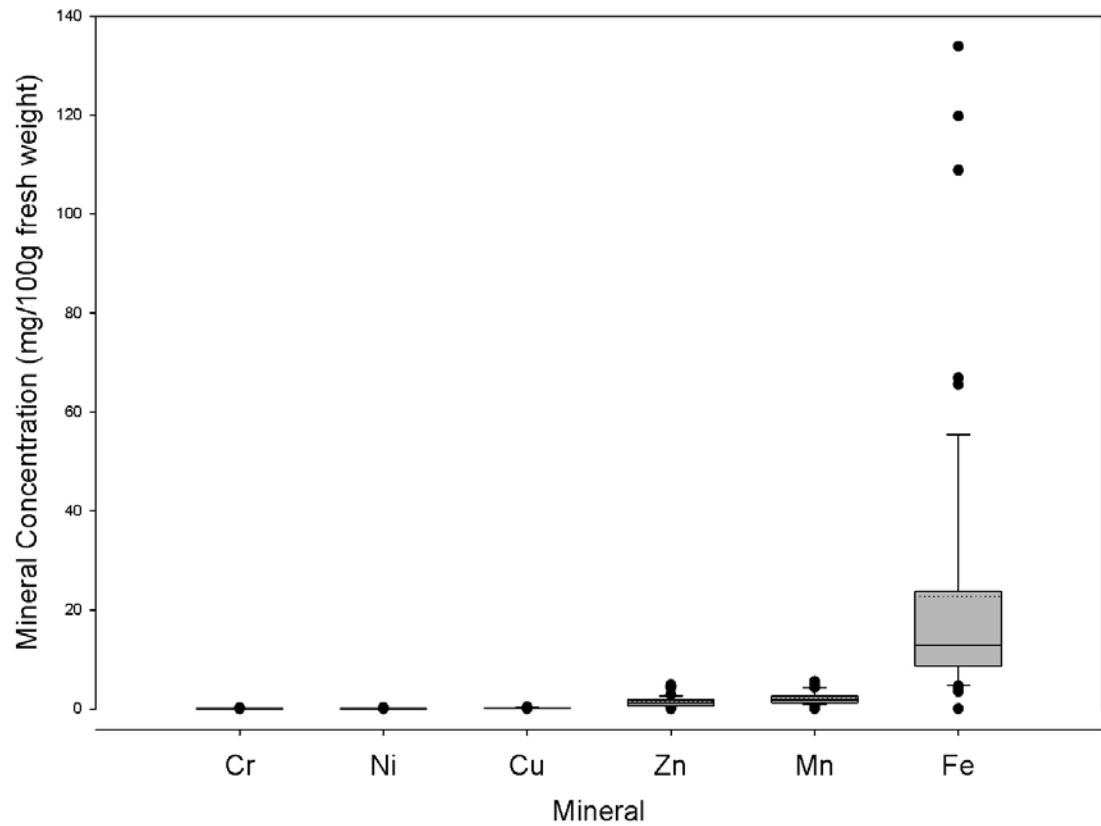


Figure 3. Trace mineral content of *C. lanceolata* corms from all populations (n=72). Solid lines represent the median values; dotted lines represent the mean values; outliers are represented as solid circles.

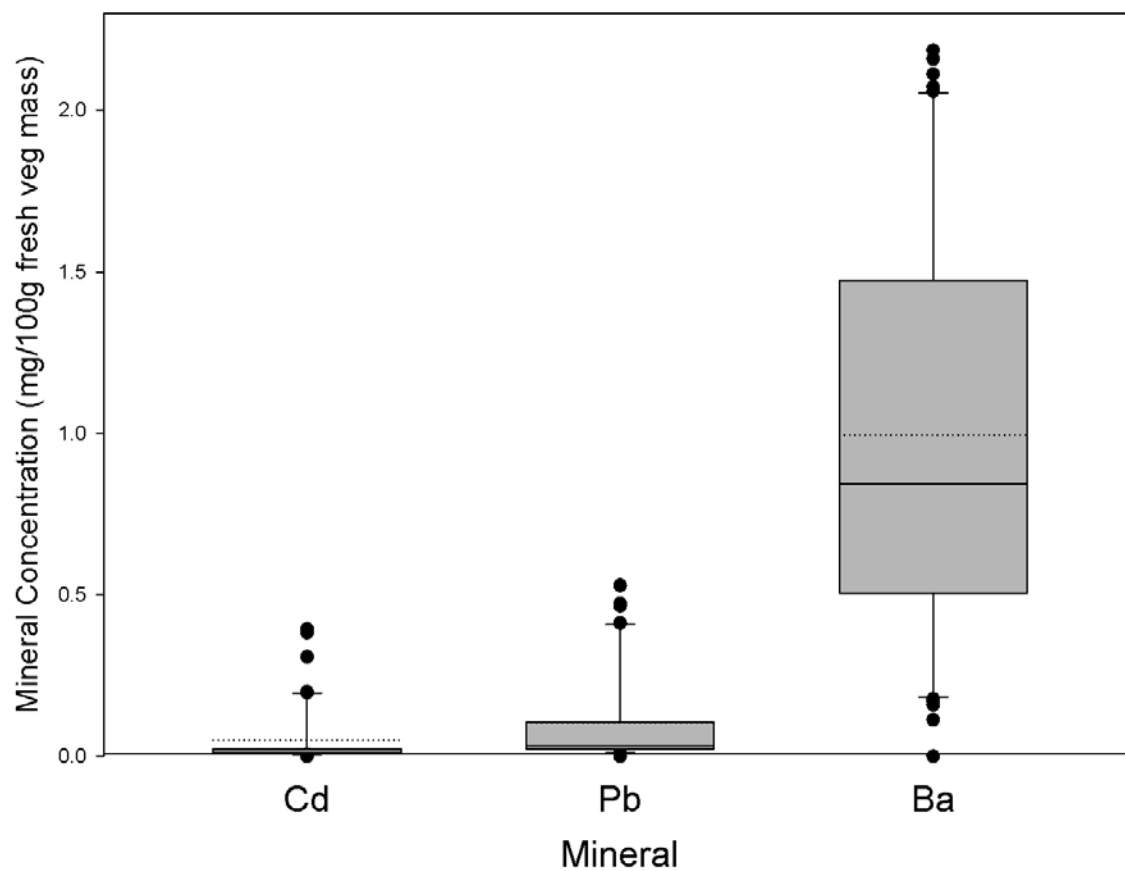


Figure 4. Toxic metal content of *C. lanceolata* corms from all populations (n=72). Solid lines represent the median values; dotted lines represent the mean values; outliers are represented as solid circles.

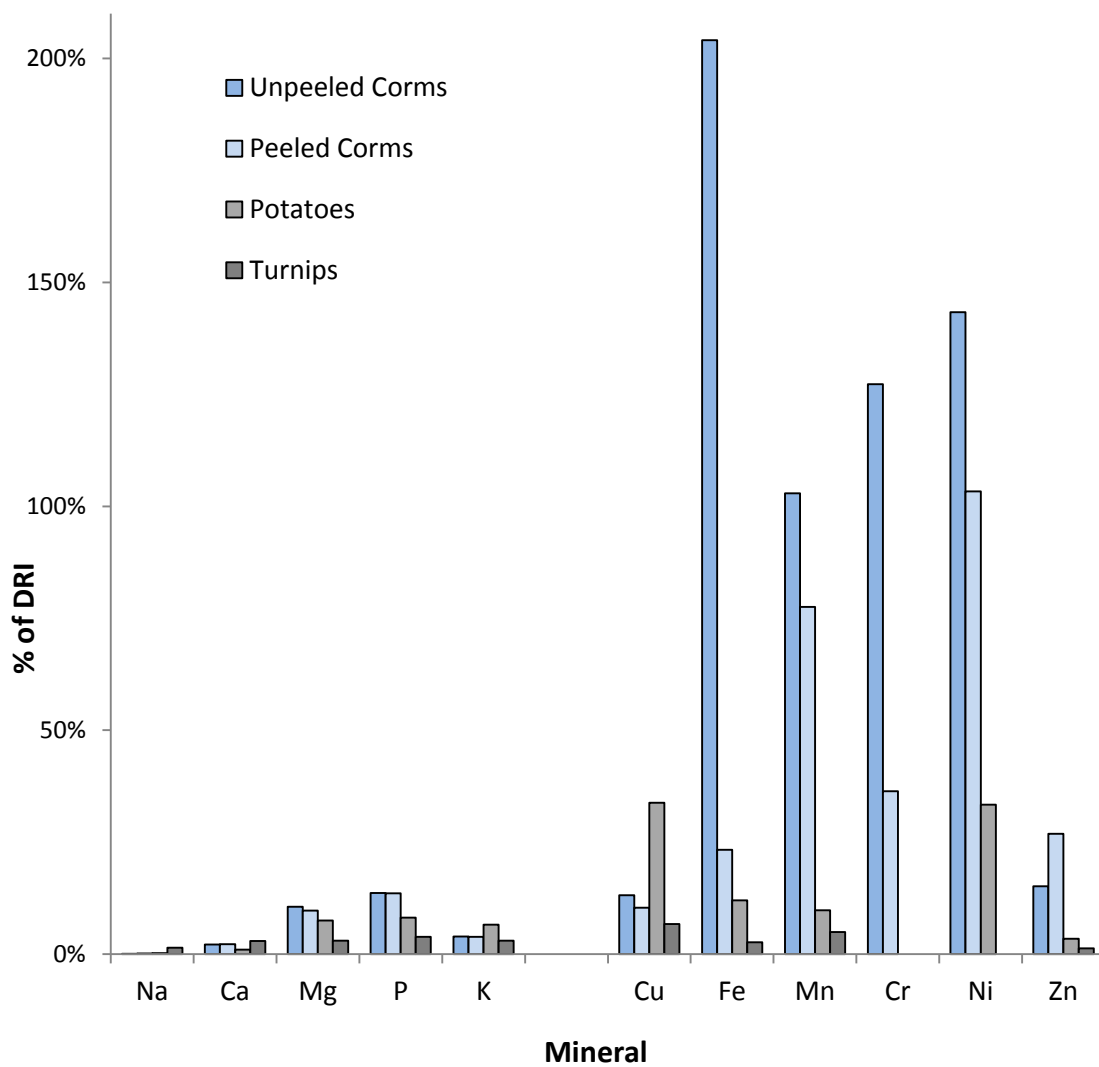


Figure 5. Percentage of DRI for dietary minerals in a 100g serving of various plant foods.

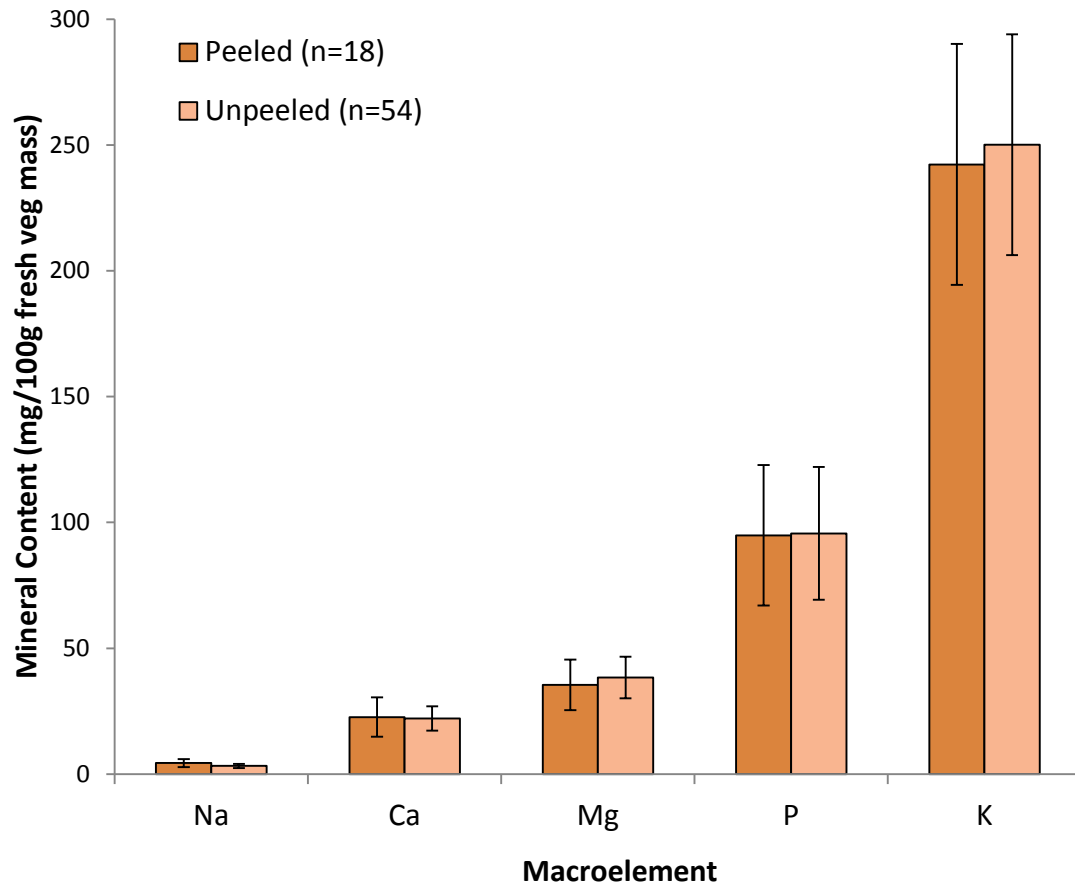


Figure 6. Mean macroelement content of peeled *C. lanceolata* corms compared to unpeeled corms. Error bars represent  $\pm 1$  standard deviation.

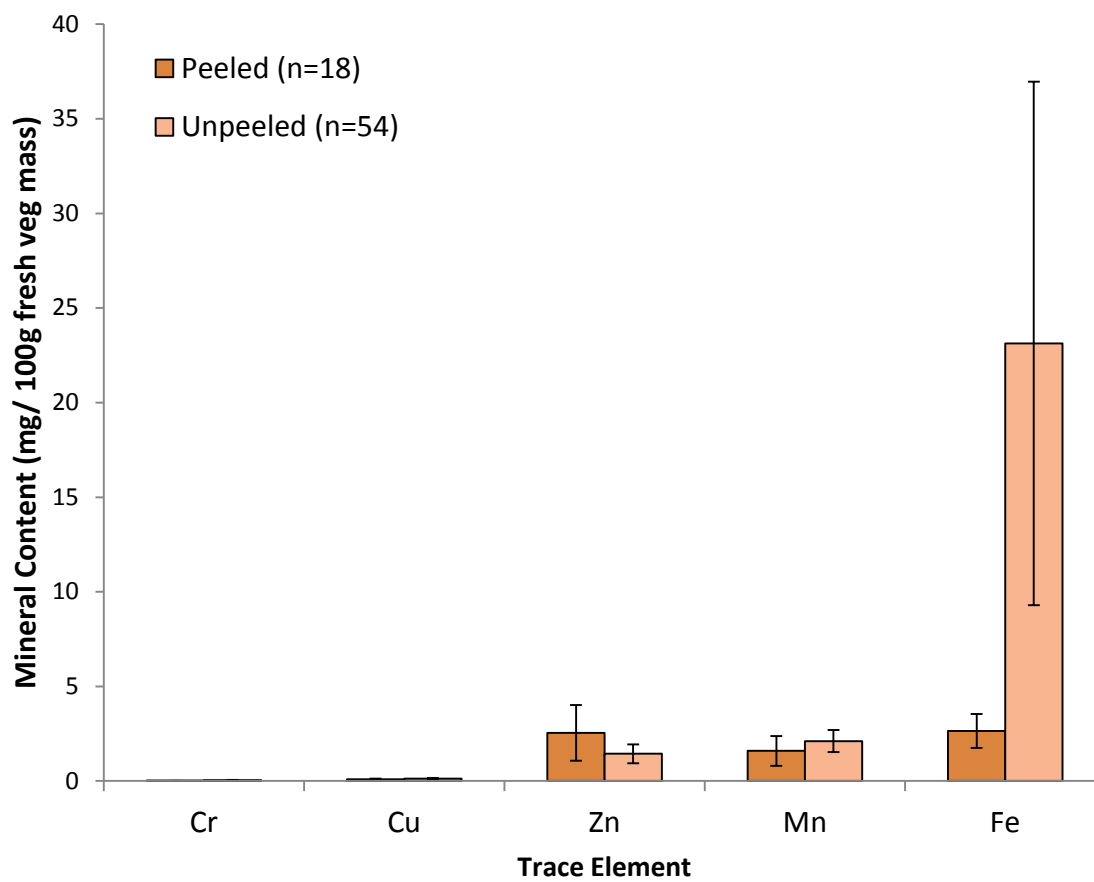


Figure 7. Mean trace element content of peeled *C. lanceolata* corms compared to unpeeled corms. Error bars represent +/- 1 standard deviation.

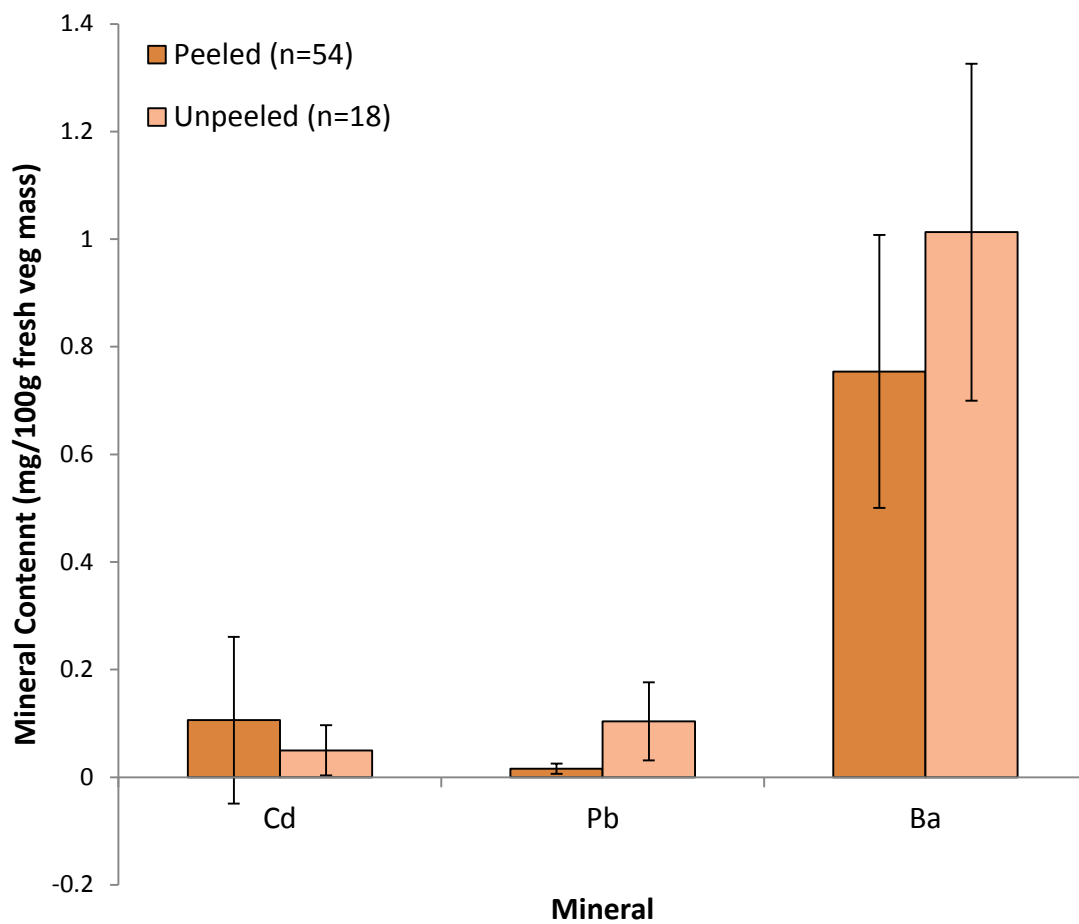


Figure 8. Mean toxic metal content of peeled *C. lanceolata* corms compared to unpeeled corms. Error bars represent +/- 1 standard deviation.

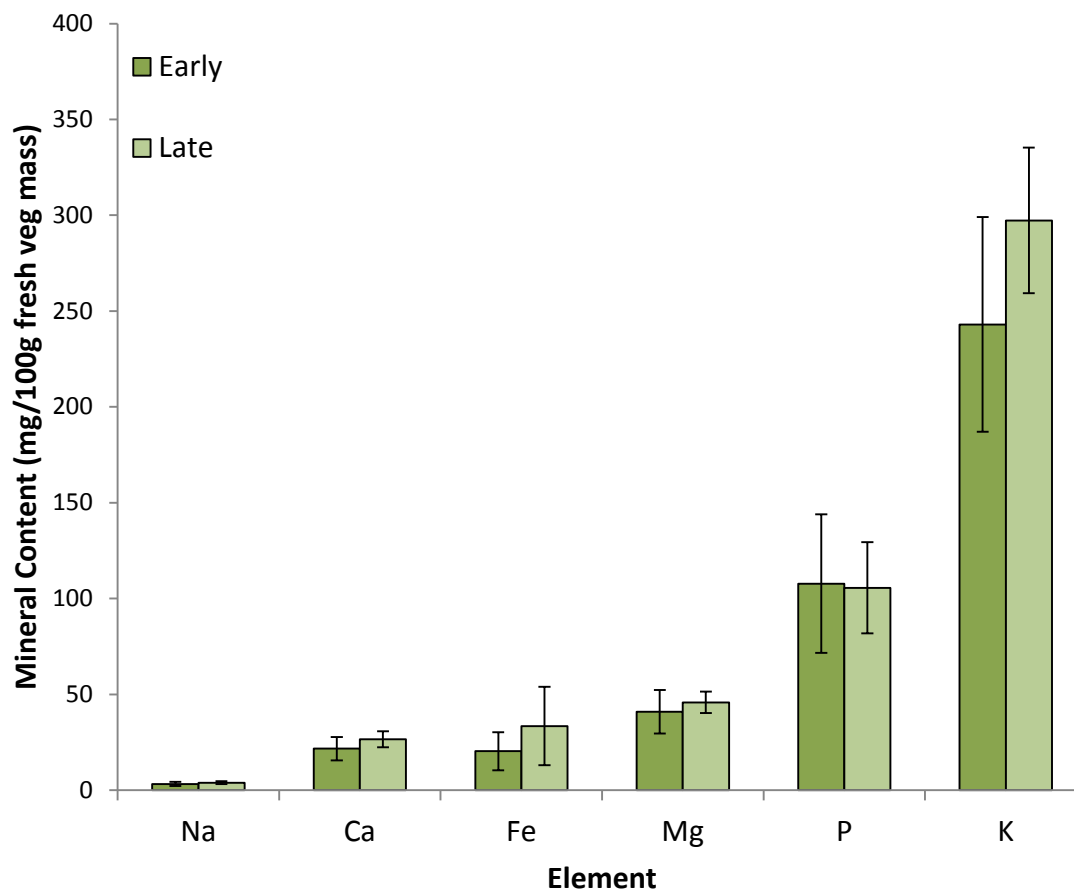


Figure 9. Average dietary mineral content of *C. lanceolata* corms from populations sampled ~30 days apart (n=18). Error bars represent +/- 1 standard deviation. Iron (Fe) is added to this graph due to its high average concentrations.

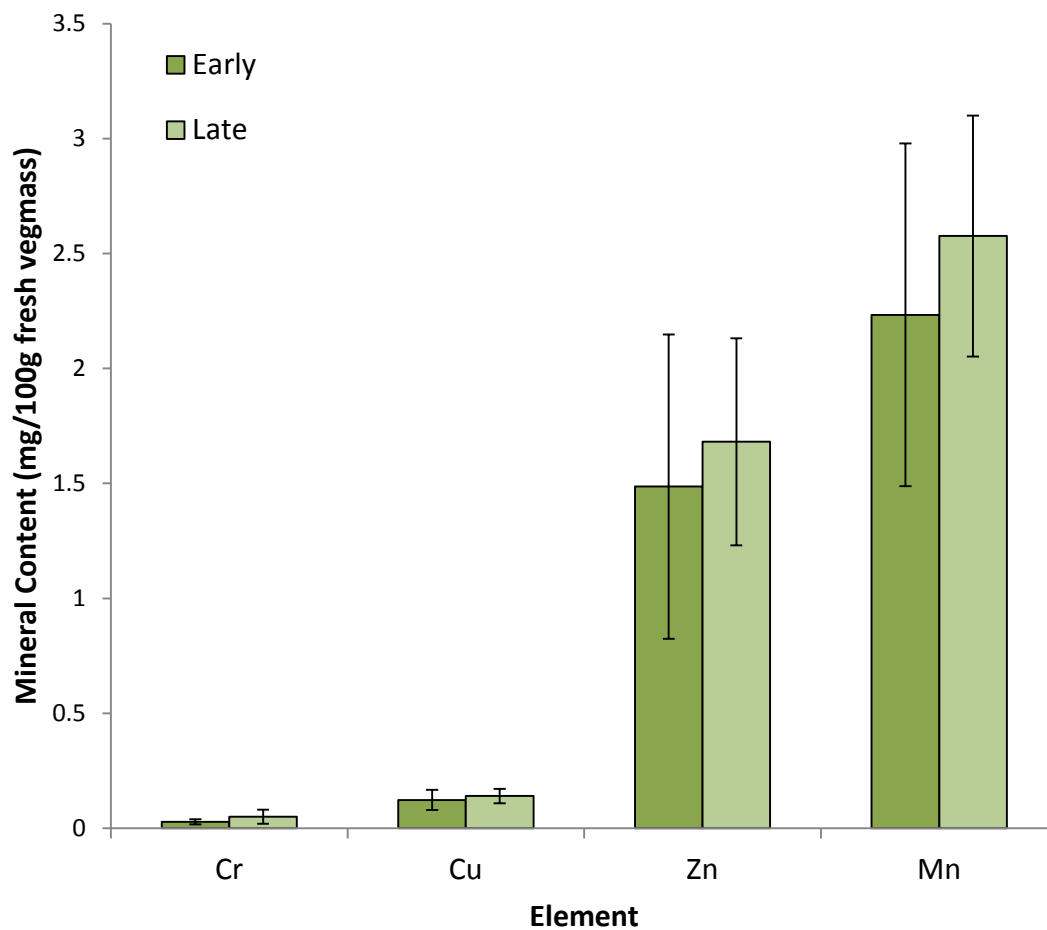


Figure 10. Mean dietary mineral content of *C. lanceolata* corms from populations sampled ~30 days apart (n=18). Error bars represent +/- 1 standard deviation.



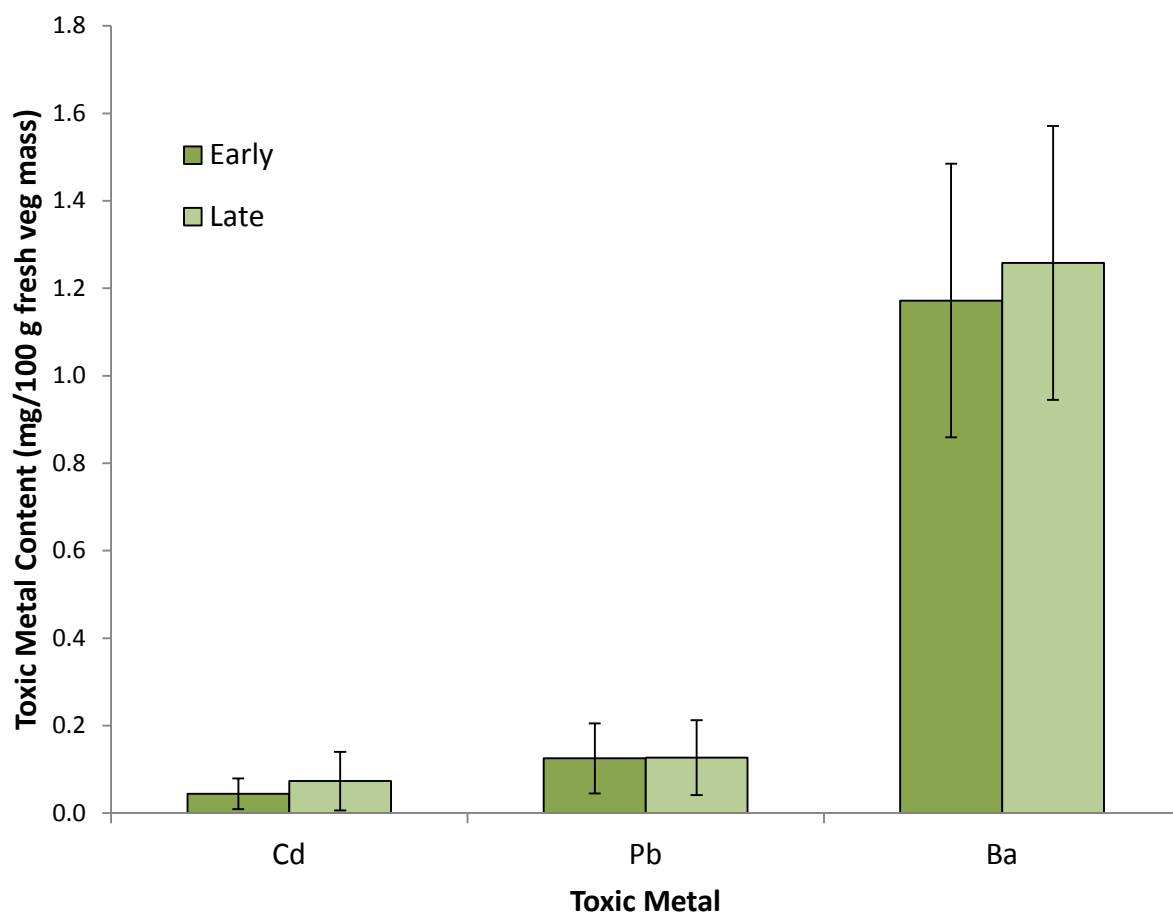


Figure 11. Mean toxic metal concentration in *C. lanceolata* corms from populations sampled ~30 days apart (n=18). Error bars represent +/- 1 standard deviation.

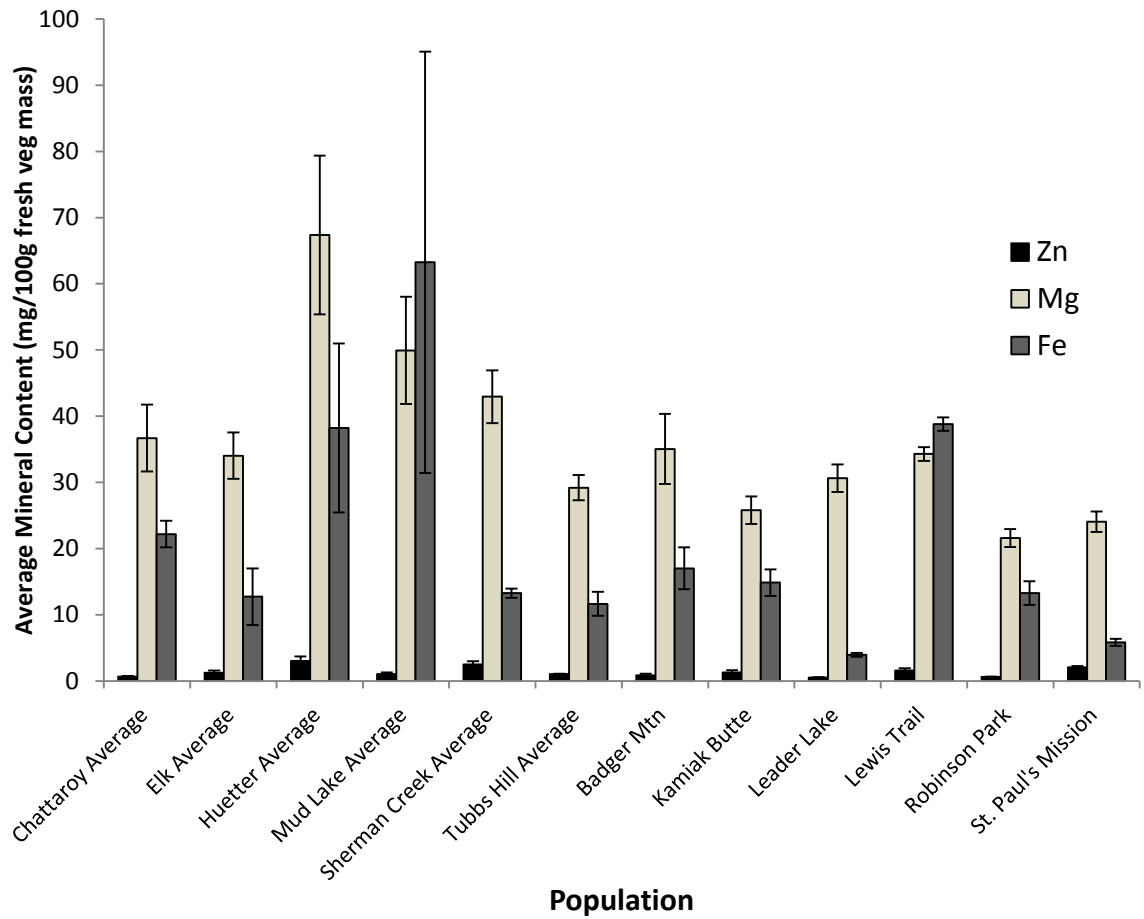


Figure 12. Mean Fe, Mg, Zn content of *C. lanceolata* corms from various populations (n=3).

Table 1. Summary of benefits and symptoms of deficiency associated with the mineral nutrients important to human health analyzed in this study.

	Mineral	DRI* (mg/day)	Function	Symptoms of deficiency	Toxicity Level
Macroelement	Ca	1000	Biological function of numerous tissues, important cofactor, physiological performance in general	Muscle cramps, brain function, osteoporosis	-
	Mg	365	Energy metabolism, release of neurotransmitters, cell function	Nausea, irritability, muscle weakness, twitching, cramps, cardiac arrhythmias	>5000mg/day
	K	6350	Maintain fluid balance, assists nerve function, related to heart muscle contraction	Cramping, muscle weakness, mood changes and irregular heartbeat	-
	Na	4700	Maintains the balance of fluids, and is related to blood pressure, kidney function, nerve and muscle function	Nausea, dizziness, poor concentration, and muscle weakness	-
	P	700	Required to produce ATP as a source of energy, and may regulate numerous protein activities	Sore bones, irregular breathing, anxiety, fatigue, and changes in body weight	-
Trace Element	Cu	0.9	Related to enzyme function and the development of connective tissue and nerve coverings	Hematological symptoms and skeletal disturbances	>2.6 mg/day
	Fe	11.3	Synthesis of hemoglobin and myoglobin needed for O <sub>2</sub> transport, and energy release	Weakness, fatigue, headaches, shortness of breath, difficulty concentrating	10-20 mg/kg
	Mn	2.05	Enzyme cofactor involved in antioxidant reactions	Reduction in red blood cells, cholesterol	0.16 mg/kg
	Cr	0.028	Essential for normal blood glucose and lipid metabolism	Glucose intolerance and weight loss	1.9 -3.3 mg/kg
	Zn	9.5	Required in the production and activity of over 100 enzymes , in the synthesis of nucleic acids, for cellular differentiation, and insulin secretion	Impairs DNA synthesis, dulls the sense of taste and smell, affects the immune system, and can cause hair loss	>225 mg/day

Table 2. Summary of the detrimental effects of some metals toxic to human health.

Toxic Metals	Metal	Effect on Human Health
	<b>Cadmium (Cd)</b>	Increased cancer risk, impaired bone metabolism, and poor endocrine system function
	<b>Barium (Ba)</b>	Not well understood. Some animal studies show increased swelling, changes in organ weights, and decreased survival
	<b>Lead (Pb)</b>	Acute symptoms include headaches, nausea, birth defects, and miscarriage. Chronic affects include anemia, infertility, kidney damage, and hypertension.

Table 3. Sampling/resampling dates, GPS coordinates, elevation, culture area and soil data for *C. lanceolata* corm collection sites.

Sample Site Name	Sample Date	Resample Date	Latitude**	Longitude**	SSURGO MUKEY*	Elevation (m)	Culture Area
St. Paul's Mission	5/3/2014	-	48°37'56.995"N	118°6'10.634"W	158261	421	Coeur d'Alene
Huetter Rest Area	4/11/2014	5/16/2004	47°42'47.53"N	116°52'35.496"W	79439	658	Coeur d'Alene
Tubbs Hill	4/11/2014	5/16/2014	47°40'17.785"N	116°47'10.35"W	79453	698	Coeur d'Alene
Lewis Trail	4/4/2014	-	46°48'47.508"N	116°58'17.755"W	1387525	949	Coeur d'Alene
Robinson Park	4/5/2014	-	46°45'4.857"N	116°54'35.286"W	1689277	839	Coeur d'Alene
Sherman Creek	4/19/2014	5/17/2014	48°35'41.228"N	118°9'35.99"W	70171	572	Colville
Mud Lake	4/19/2014	5/18/2014	48°16'11.72"N	117°39'56.244"W	158233	826	Colville
Elk, WA	4/20/2014	5/18/2014	48°24'1.198"N	117°15'24.531"W	621133	590	Kalispel
Leader Lake	5/3/2014	-	48°22'4.494"N	119°41'43.469"W	1899793	432	Mid-Columbia River Salishian
Badger Mountain	5/2/2014	-	47°36'4.097"N	120°8'14.973"W	704240	1125	Mid-Columbia River Salishian
Kamiak Butte	4/13/2014	-	46°51'31.403"N	117°10'50.724"W	68481	1094	Sanpoil
Chattaroy, WA	4/11/2014	5/15/2014	47°53'42.698"N	117°23'57.919"W	620934	601	Spokane

\*SSURGO MUKEY is the reference number for the information on the soil type as collected by the National Cooperative Soil Survey of the USDA-NCRS. The MUKEY allows interested parties to query the database for detailed soil information.

\*\*Latitude and Longitude are based on the WGS-84 Coordinate system.

Table 4. Population averages for moisture, mineral, and toxic metal content of *C. lanceolata* corms from all populations and sample times.

Population (n=3)	Collection Date 2014	% Water	Concentration (mg/100g fresh weight)													
			Macroelements					Trace Elements					Heavy Metals			
			Ca*	Mg*	K*	Na*	P*	Cu	Fe*	Mn*	Cr	Zn*	Cd*	Pb*	Ba*	Al*
<b>Chattaroy</b>	4/1	83	14.5	<b>28.1</b>	<b>156.2</b>	2.8	78.1	<b>0.08</b>	24.3	1.8	0.03	0.5	0.01	<b>0.03</b>	1.7	17.7
<b>Chattaroy</b>	5/5	69	20.9	<b>45.3</b>	<b>275.7</b>	2.4	75.5	<b>0.11</b>	20.1	1.9	0.02	0.8	0.01	<b>0.02</b>	1.9	14.0
Elk	4/20	78	18.1	29.9	206.9	2.8	83.0	0.08	10.3	1.9	0.02	0.8	0.01	0.03	1.1	9.5
Elk	5/16	66	28.0	38.2	303.2	3.9	95.6	0.09	15.1	2.1	0.03	1.7	0.01	0.05	1.1	13.4
<b>Huetter</b>	4/11	69	<b>46.8</b>	<b>89.0</b>	<b>475.9</b>	<b>7.7</b>	<b>253.8</b>	<b>0.31</b>	<b>60.4</b>	<b>5.1</b>	<b>0.07</b>	4.1	<b>0.03</b>	<b>0.18</b>	<b>2.1</b>	<b>44.7</b>
<b>Huetter</b>	5/16	72	<b>21.7</b>	<b>45.8</b>	<b>292.2</b>	<b>5.4</b>	<b>109.0</b>	<b>0.11</b>	<b>16.0</b>	<b>1.7</b>	<b>0.02</b>	2.0	<b>0.02</b>	<b>0.04</b>	<b>1.3</b>	<b>16.0</b>
<b>Mud Lake</b>	4/19	81	<b>18.0</b>	<b>35.3</b>	<b>214.3</b>	<b>2.5</b>	<b>53.2</b>	<b>0.08</b>	<b>5.6</b>	<b>0.8</b>	<b>0.01</b>	<b>0.6</b>	<b>0.01</b>	<b>0.01</b>	<b>1.1</b>	<b>6.4</b>
<b>Mud Lake</b>	5/18	64	<b>38.8</b>	<b>64.6</b>	<b>434.9</b>	<b>6.0</b>	<b>202.3</b>	<b>0.26</b>	<b>120.9</b>	<b>4.4</b>	<b>0.18</b>	<b>1.5</b>	<b>0.02</b>	<b>0.09</b>	<b>2.0</b>	<b>77.2</b>
<b>Sherman Creek</b>	4/11	77	16.4	36.5	209.8	<b>1.8</b>	<b>51.7</b>	<b>0.09</b>	12.4	2.6	0.03	1.8	<b>0.19</b>	0.45	0.3	10.5
<b>Sherman Creek</b>	5/17	69	28.0	49.4	277.9	<b>3.3</b>	<b>86.5</b>	<b>0.15</b>	14.1	3.2	0.03	3.2	<b>0.36</b>	<b>0.49</b>	0.4	12.8
<b>Tubbs Hill</b>	4/11	83	16.2	26.8	195.1	2.0	<b>126.9</b>	0.11	<b>8.9</b>	<b>1.2</b>	<b>0.01</b>	1.0	0.02	<b>0.05</b>	0.7	<b>6.7</b>
<b>Tubbs Hill</b>	5/16	76	22.5	31.6	199.7	2.5	<b>64.9</b>	0.12	<b>14.4</b>	<b>2.2</b>	<b>0.02</b>	1.0	0.02	<b>0.08</b>	0.8	<b>13.5</b>
Badger Mountain	5/2	84	21.6	35.0	248.2	2.0	84.3	0.09	17.0	1.5	0.02	0.9	0.01	0.02	1.1	11.1
Kamiak Butte	4/3	75	18.2	25.8	183.5	2.7	55.0	0.08	14.9	1.5	0.02	1.3	0.01	0.02	0.6	10.0
Leader Lake	5/3	81	13.0	30.6	197.6	2.6	62.7	0.08	4.0	0.7	0.01	0.5	0.01	0.01	0.2	3.7
Lewis Trail	4/4	78	22.8	34.3	257.0	3.0	98.4	0.10	38.8	2.3	0.05	1.6	0.01	0.03	1.0	27.7
Robinson Park	4/4	74	14.9	21.6	171.2	1.9	80.4	0.09	13.3	1.6	0.03	0.6	0.01	0.05	0.7	15.0
St. Paul's Mission	5/3	78	17.0	24.1	203.2	2.9	60.0	0.10	5.8	1.6	0.02	2.0	0.15	0.24	0.2	2.9
Average	n/a	75	22.1	38.4	250.1	3.2	95.6	0.12	23.1	2.1	0.03	1.4	0.05	0.10	1.0	17.4

-Light shading represents the corms that had between 10-25% of the DRI of the mineral per 100g serving; medium shading represents between 25-50% of DRI; dark shading represents 50-75% of DRI; outlined boxes represent populations that contained >100% of DRI.

-Double outlined boxes represent populations with average sample concentrations over the EURL for toxic metals.

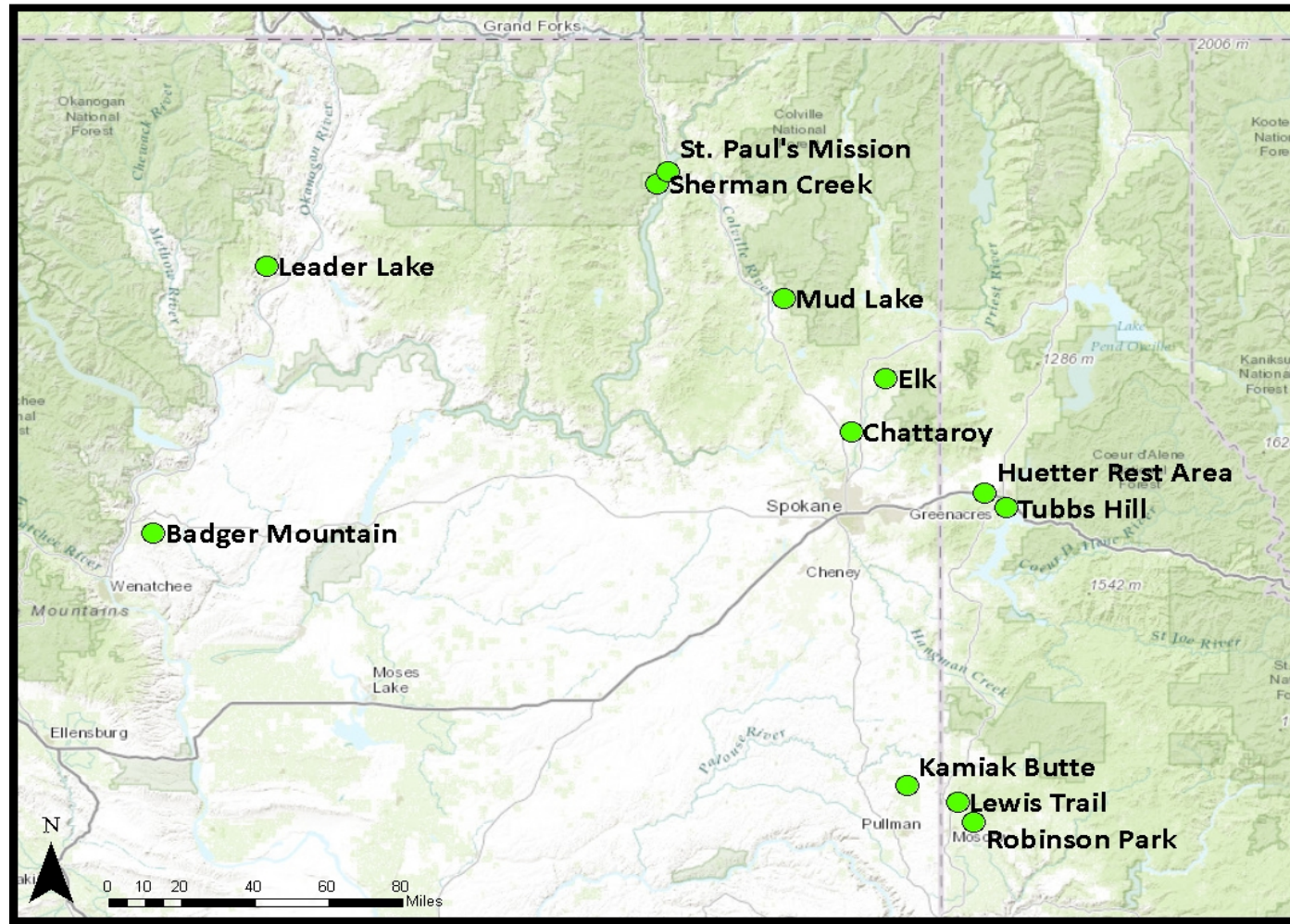
-The average minerals content of corms that differed significantly ( $p < .05$ ) between populations are denoted with an asterisk (\*) after the element.

-Populations and elements listed in bold include significantly different average concentrations of elements between harvest times.

Table 5. Percent of recovery for each element from check standards CRM-Soil B and CRM – OL from High Purity Standards.

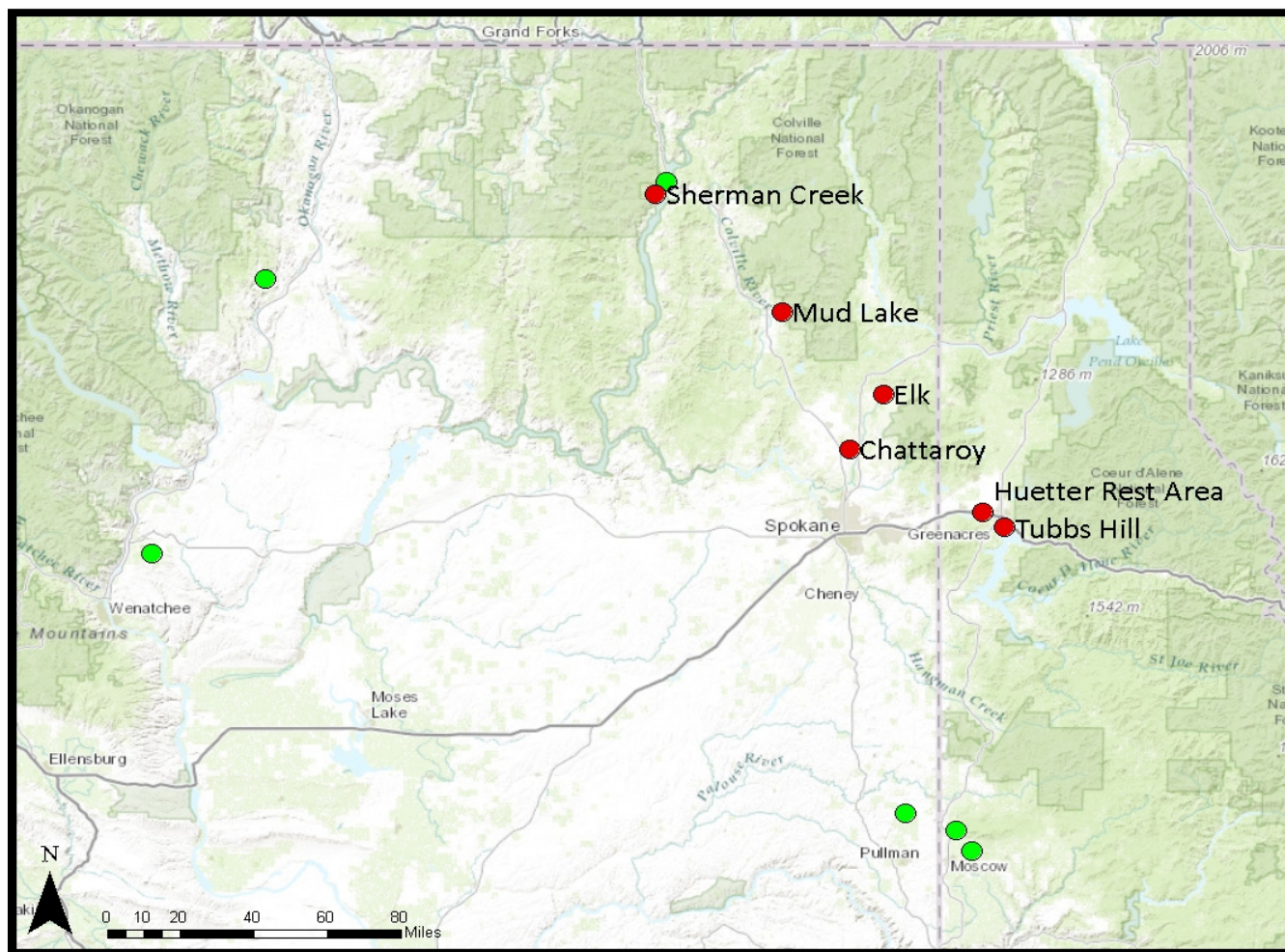
<b>Standard</b>	<b>Al</b>	<b>As</b>	<b>Ba</b>	<b>Ca</b>	<b>Cd</b>	<b>Cr</b>	<b>Cu</b>	<b>Fe</b>	<b>K</b>	<b>Mg</b>	<b>Mn</b>	<b>Na</b>	<b>P</b>	<b>Pb</b>	<b>Zn</b>
CRM-Soil B	112	108	113	104	113	125	110	104	156	100	105	100	161	113	107
CRM - OL	92	116	110	99	100	90	112	105	108	99	100	120	156	111	107

Map 1. Map of *C. lanceolata* corm collection sites.

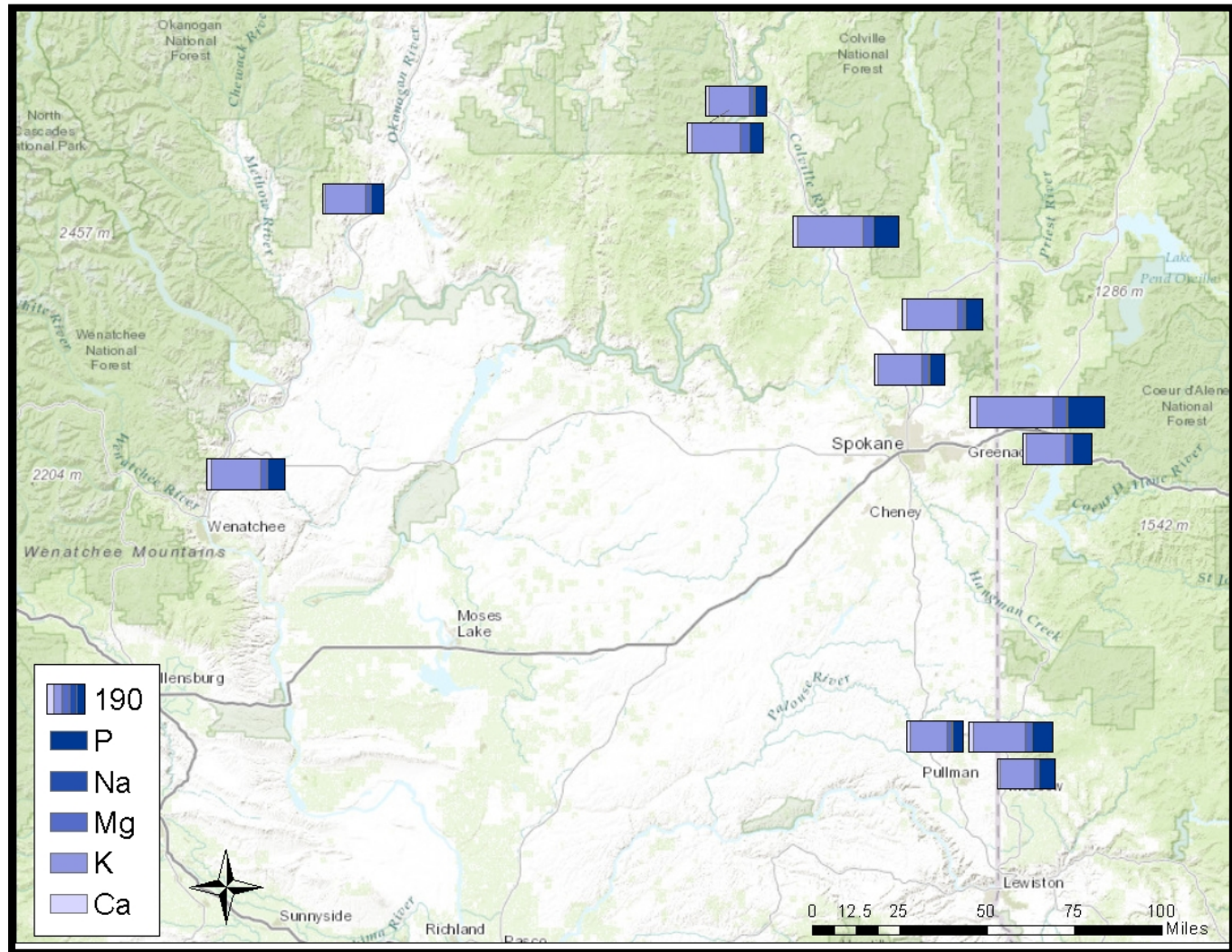




Map 2. Map of resampled *C. lanceolata* corm collection sites.

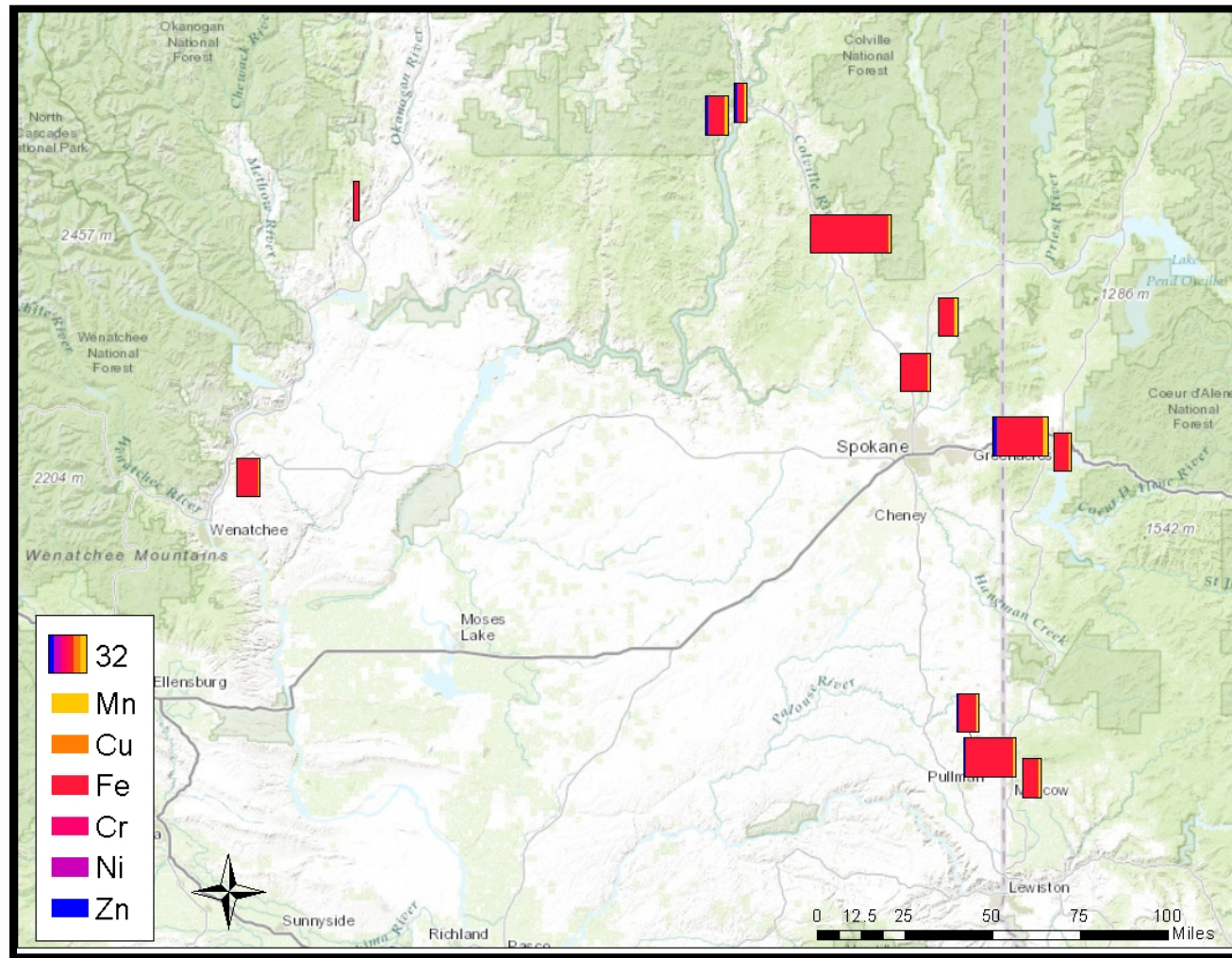


Map 3. Map of macroelement concentrations for *C. lanceolata* corm collection sites.

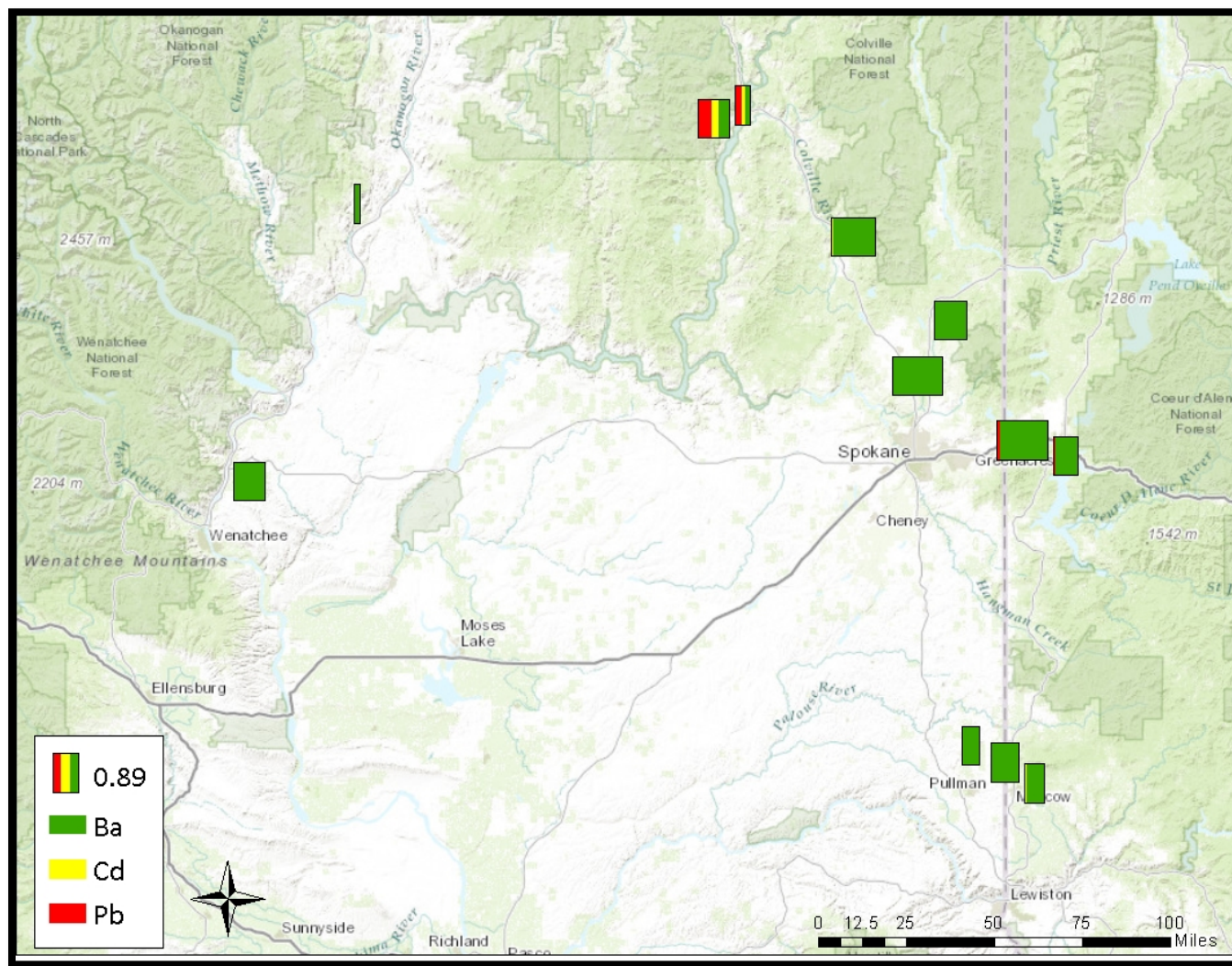




Map 4. Map of microelement concentrations for *C. lanceolata* corm collection sites.

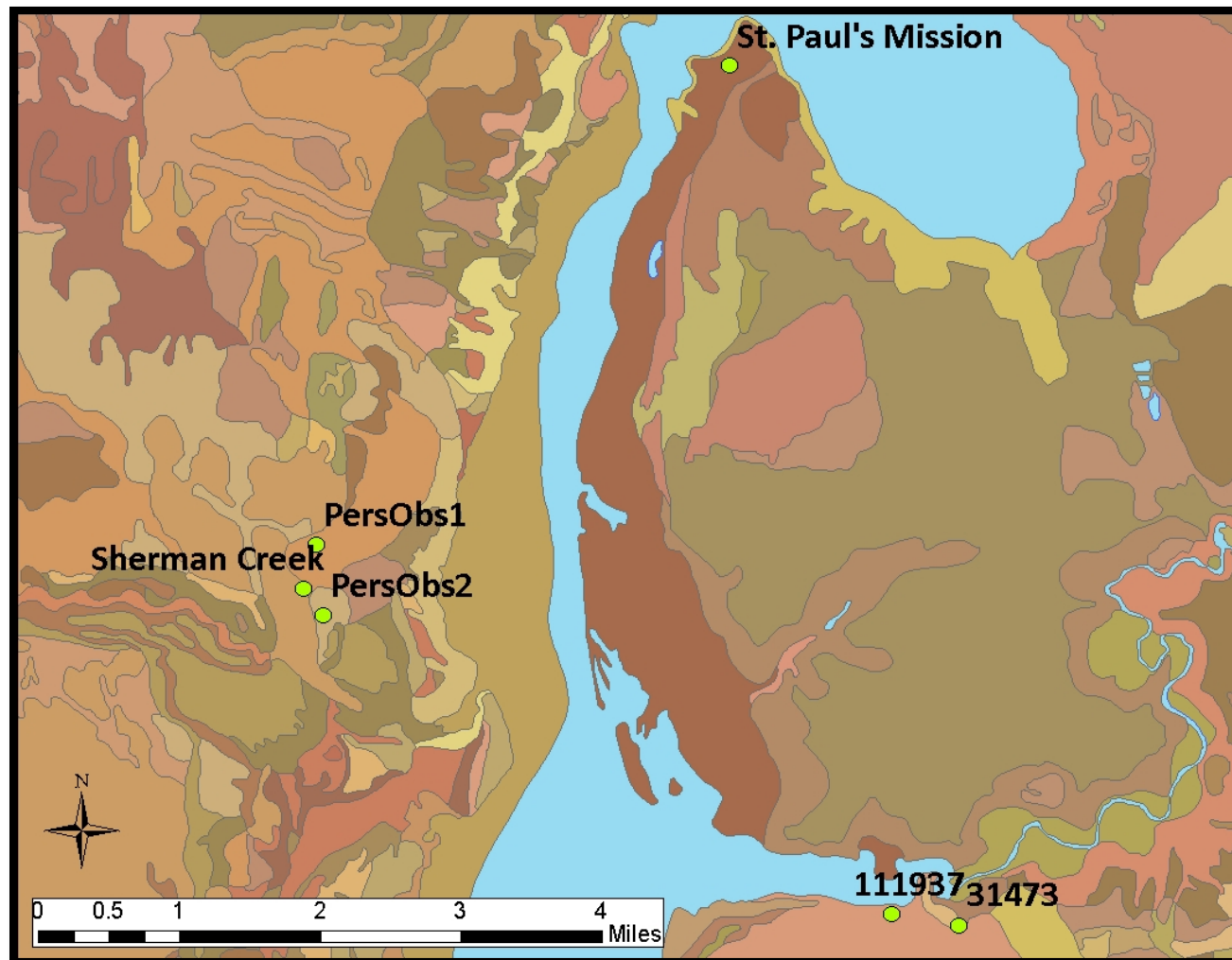


Map 5. Map of sample site mineral concentrations represented as a horizontal bar figure of toxic metals with individual metals separated by color.





Map 6. Map of SSURGO data overlain with known sites where *C. lanceolata* grows. Listed are sites sampled in this study (St. Paul's Mission and Sherman Creek), places I saw *C.lanceolata* growing and took a GPS coordinate (PersObs1 and PersObs2), and sites from PNW Consortium of Herbia records (111937 and 31473).



## **Appendix 1. Dietary Minerals Essential to Human Health**

Throughout this paper, I reference the Dietary Reference Intake (DRI). The DRI are published by the Institute of Medicine (IOM) and represent the most current scientific knowledge on the nutrient requirements of healthy populations. Individuals may need more or less of individual nutrients, but the DRI is a good point of reference for comparison of foods and health (IOM 2011). The DRI allowance of a specific nutrient is defined as the intake level that minimizes the risk of deficiency or excess (WHO/FAO 2004).

Dietary mineral nutrients are the chemical elements other than carbon (C), oxygen (O), hydrogen (H), and nitrogen (N) present in organic molecules. The dietary mineral nutrients essential to human health are generally categorized in two groups; 1) the macroelements which are required by humans in larger quantities, and include calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), and phosphorus (P) and 2) trace elements which include copper (Cu), iron (Fe), manganese (Mn), chromium (Cr) and zinc (Zn). Toxic Metals offer no benefits to human health, such as Cd, Ba, and Pb.

### *Calcium (Ca)*

Calcium content of food plants varies widely (Martinez-Ballesta et al. 2010), and is an essential nutrient for human health. Calcium participates in biological functions of various tissues (musculoskeletal, nervous and cardiac, bones and teeth, parathyroid gland), acts as a cofactor in numerous enzyme reactions, and is involved in physiological performance in general (Theobald 2005, Huskisson et al. 2007, Morgan 2008, Williams 2008). The recommended daily allowance is 800-1300 mg/day.

### *Magnesium (Mg)*

In general, food plants contain 5-190 mg/100g fresh weight of Mg. Magnesium is related to energy metabolism, release of neurotransmitters, and cell function (Bo and Pisu 2008). Additionally, Mg acts as a cofactor of up to 300 enzymes (Huskisson 2007). An increased intake of magnesium has shown to mitigate the risk of diabetes, hypertension, and

cardiovascular conditions (Bo and Pisu 2008). The recommended intake is 200-400 mg/day.

#### *Potassium (K)*

Food plants generally contain potassium from 20 to 730 mg/100g fresh weight, although some food plants contain much higher levels (many seeds and nuts, potatoes, banana, avocado). Potassium helps maintain the balance of the physical fluid system, assists nerve function, and is related to heart muscle contraction (Rosenthal and Gilly 2003, Schwarz and Bauer 2004, Ko et al. 2008, Lambert et al 2008). Symptoms of deficiency include cramping, muscle weakness, mood changes and irregular heartbeat (Sobotka et al 2008). Recommended intake is around 3500 mg/ day.

#### *Sodium (Na)*

Food plants generally contain low levels of Na (between 2 and 94 mg/100g fresh weight). Na helps maintain the balance of fluids, and is related to blood pressure, kidney function, nerve and muscle function (Martinez-Ballesta et al. 2010). Deficiencies are rare, but symptoms can include nausea, dizziness, poor concentration, and muscle weakness (Smith et al. 2000). Recommended intake is 2400 mg/day.

#### *Phosphorus (P)*

Generally present in food plants in the range of 16-440 mg/100g fresh weight, phosphorus is required to produce ATP, GTP and CP as a source of energy, and may regulate numerous protein activities (Sobotka et al 2008). Symptoms of deficiency include sore bones, irregular breathing, anxiety, fatigue, and changes in body weight (Martinez-Ballesta et al. 2010). The recommended daily intake of P is 800-1300 mg/day.

#### *Copper (Cu)*

Generally, copper is present in food plants in low levels (.004 to .5mg/ 100g fresh weight). Primarily, Cu is related to enzyme function and the development of connective tissue and nerve coverings (Huskisson et al. 2007, Shenkin 2008). Cu can be stored in the adult human body at up to 80mg so deficiencies are rare, but can include haematological

symptoms and skeletal disturbances (Guerrero-Romero and Rodriguez-Moran 2005, Huskisson et al. 2007). The recommended daily uptake of Cu is between 1 and 1.6 mg/day.

#### *Iron (Fe)*

Iron contents of food plants are usually low, ranging from 0.1 to 3 mg/100g fresh weight. The main functions of Fe are the synthesis of haemoglobin and myoglobin needed for O<sub>2</sub> transport and energy release (Huskisson et al. 2007, Shenkin 2008). Symptoms of deficiency include anemia. The recommended daily intake is 8-18 mg/day.

#### *Manganese (Mn)*

Low levels of Manganese in the range of 0.01 to 0.08 mg/100g fresh weight are present in many food plants. Manganese is an enzyme cofactor involved in antioxidant reactions (Rodriguez-Moran 2005). Deficiencies are rare, but symptoms include a reduction in red blood cells, cholesterol, and other abnormalities (Shenkin 2008). The recommended daily intake of Mn is 2mg/day.

#### *Chromium (Cr)*

Chromium in food plants is generally found in trace amounts ( $4 \times 10^{-5}$  to  $6 \times 10^{-3}$  mg/100 g fresh weights). Chromium is essential for normal blood glucose and lipid metabolism (Huskisson et al. 2007) as well as gene expression, lipid synthesis, and metabolism regulation (Shenkin 2008). Deficiencies appear symptomatically as glucose intolerance and weight loss (Shenkin 2008). Recommended intake of chromium is between 25-35 µg/day.

#### *Zinc (Zn)*

Zinc concentrations in food plants generally vary from 0.05 to 12 mg/ 100 g fresh weight. Zinc is required in the production and activity of over 100 enzymes (Shenkin 2008), in the synthesis of nucleic acids, for cellular differentiation, and insulin secretion (Lukaski 2004). Zn deficiency is relatively frequent, and impairs DNA synthesis, dulls the sense of



taste and smell, affects the immune system, and can cause hair loss (Shenkin 2008). The recommended intake of Zn is 8-11mg daily.

## **Appendix 2. Select Toxic Metals Detrimental to Human Health**

One problem with foraged foods in modern times is the presence of environmental contaminants such as toxic metals. A summary of some toxic metals and their detriment to human health is outlined below.

### *Cadmium (Cd)*

Cadmium in certain forms is highly toxic to humans. It is used in a number of industrial processes is readily taken up by root plants (carrots, parsnips), leafy vegetables (spinach), and grains (wheat), and Cd accumulates in the body with many toxic effects, such as increased cancer risk, impaired bone metabolism, and poor endocrine system function (Mudgal et al. 2010).

### *Barium (Ba)*

Barium is a potentially toxic metal that is sometimes found naturally in the environment, and occasionally in foods. Information is being collected on how exposure to barium affects human health. Animal studies show increased swelling and irrational, changes in organ weights, and decreased survival (DHS).

### *Lead (Pb)*

Lead is a naturally occurring heavy metal used by people since ancient times in a number of products. Inhalation or ingestion of lead is associated with a number of acute and chronic adverse health effects. Acute symptoms include headaches, nausea, birth defects, miscarriage, and others. Chronic affects include anemia, infertility, kidney damage, and hypertension. The effects are especially dangerous to children, as lead affects the development of the nervous system, and can cause permanent learning and behavioral problems. Lead can stay in the body for years and is stored in bone and soft tissue, and can be re-released into the bloodstream during times of high calcium demand, such as pregnancy, menopause, and aging (NIH 2015).

## **Vita**

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