CPS Rapid Response
FINAL PROJECT REPORT

Project Title
Expanded Sampling and Mitigation Strategy Evaluation for Cadmium in Desert Spinach

Project Period
January 10, 2016 – July 9, 2016 (as per contract)

Principal Investigator
Paul Brierley, Executive Director
Yuma Center of Excellence for Desert Agriculture

Co-Principal Investigators
Dr. Charles A. Sanchez, Professor
Soil, Water, and Environmental Sciences, University of Arizona

---

Objectives

The objectives of the project were to expand current sampling efforts during the current Yuma growing season to develop tools and management strategies to reduce heavy metals in vegetable crops. Studies included the development of a soil test that growers can use to predict the potential for a given field to produce crops with problematic levels of Cd. This evaluation included soil test calibrations and high resolution soil sampling to assess the in-field variability and guide sampling protocols. Studies for evaluation of Zn fertilization as a potential strategy to reduce Cd accumulations in leafy vegetable crops were also performed.

While some of this work was in progress with Specialty Crop Block Grant funding, CPS Rapid Response funding allowed the following:

- The collection and analysis of high resolution soil samples to assess in-field variability;
- Doubling of the soil test calibration database, creating more reliability and confidence in the results;
- Evaluation of Zn fertilization on additional sites from the original plan (5 versus 2). We focused on sites where the soil has higher background Cd, and tried various rates.

Funding for this project provided by the Center for Produce Safety through:
CPS Campaign for Research Funds

FINAL REPORT

Abstract

The production of vegetable crops in the low desert region of the southwestern United States is over a 2 billion dollar industry and product is shipped nationally during the fall-winter-spring period. Inorganic contaminants, such as heavy metals, are of concern in edible fresh fruits and vegetables. Many of the alluvial soils used for crop production in the low desert contain low levels of several metals, including cadmium (Cd). We are particularly concerned about spinach which has a propensity to accumulate heavy metals. The large number of spinach samples we collected to date indicate 50% of the spinach produced in the desert would exceed the EU Maximum Levels (ML) of 200 ug/kg fresh weight (FW). Studies have shown soil tests to be potentially useful for predicting Cd accumulation by plants, however, proper sampling protocols and a predictive soil test need to be calibrated for desert soils. The objectives of these studies was to collect high resolution soil samples from typical production blocks in the desert to assess in-field variability and develop sampling protocols. We also collected paired soil and spinach tissue samples to evaluate the important soil properties affecting Cd concentrations of spinach. These data were needed as a prerequisite to developing soil testing as a management tool. The observed generally low in-field variation and normal distribution of observed values for Cd suggest that a good composite representative soil sample can be collected from the 3 to 10 ha spinach production blocks typically used in the region. While the DPTA soil test was among the best evaluated, it was not sufficiently predictive of spinach Cd concentrations to be utilized as a management tool alone. Data show that in addition to soil test Cd, soil test Zn, soil test P, and soil salinity were correlated to spinach Cd concentrations and need to be incorporated into any soil test algorithm used to predict spinach Cd concentrations. While spinach Cd concentration were negatively correlated to soil test Zn levels, the application of high rates of Zn fertilizer to mitigate Cd uptake was only marginally effective. However, modest rates of Zn fertilization to correct Zn nutrient deficiency appear justified.

Background

The production of vegetable crops in the low desert is over a 2 billion dollar industry. Most of these vegetables are irrigated with water and grown on soils that contain low levels of several heavy metals. Consumers of produce are increasingly seeking assurances from industry that their product is safe. Growing trends in monitoring and regulating heavy metals in food are producing similar concerns. Spinach presents a particular concern due to its propensity to accumulate contaminants.

Cadmium in excessive amounts can cause hypertension, kidney impairment, genetic toxicity, immunotoxicity, neurotoxicity, and carcinogenicity (ATSDR, 1990). Cadmium (Cd) is naturally present in many soils and in most phosphate fertilizers (Mortvedt, 1981; 1987). Food is the major source of Cd exposure to humans (Gartrell et al., 1986; Gunderson. 1988; Pennington et al., 1986). Adult exposure to Cd has been estimated to range from 4 to 84 μg per day (Hallenback, 1984). The World Health Organization (WHO) has established a provisional daily intake of cadmium at 1 μg/kg body weight (Walker and Herman, 2000). Based on consumption estimates and cumulative exposure projections, the EU has recommended maximum levels (MLs) for various food commodities. For example, the ML for fruits, rooting vegetables, wheat, and leafy vegetables are 50, 100, 200, and 200 μg/kg FW Cd, respectively (Berg and Licht

2002). The levels of Cd in Colorado River water are generally less than 1 μg/L. However, we have found levels of Cd in phosphate fertilizers used in the low desert as high as 150 mg/kg.

Studies have shown soil tests to be potentially useful for predicting Cd accumulation by plants (Oliver et al., 1994; Norvell et al., 2000). The development of a reliable pre-plant soil test for Cd in the desert would be a valuable tool for making management decisions which could limit Cd accumulation in leafy vegetables. In 2013-2014 we compared a number of potential soil tests as predictive tools of Cd uptake in spinach. These included the Mehlich I (MI), Mehlich III (MIII), the ammonium bicarbonate-DPTA (AB-DPTA), and the DPTA extractants, as well as total soil Cd after acid digestion. While the M I (dilute acid) test proved not suitable, the DPTA, M III, AB-DPTA, the DPTA, and total soil Cd provided similar reasonable correlations. Of these better performing tests, we moved forward with the DPTA test for logistical and economic reasons.

While the DPTA test was among the best we evaluated, relationships between extractable Cd and plant concentrations are less than perfect. We suspect some combination of soil properties, in addition to plant-available soil Cd, interacts to affect plant Cd accumulation. Some studies have shown more complex models, including some measure of soil Cd as well as other soil properties such as pH (Adams et al., 2004) or soil chloride (Norvell et al., 2000), were required to predict Cd concentrations in plants. We had no information regarding soil properties affecting plant Cd concentrations in the desert to modify our soil test algorithm.

As noted above, soil pH can affect Cd availability thereby increasing plant uptake (Tiller et al., 1979; Alloway et al., 1984; Christensen, 1984; King, 1988). However, in the desert, soil pH is buffered to a narrow range by calcium carbonate and we do not believe soil pH is likely to emerge as an important variable affecting plant Cd concentration in the desert.

Soil texture can also affect Cd solubility and plant uptake since increased soil texture increases exchangeable Cd. However, increased soil texture also increases potential reactions to non-exchangeable Cd which is generally not available for plant uptake (Forbes et al, 1976; Abd-Elfattah and Wada, 1981). Many authors (Street et al. 1977; Gusenleitner et al. 1982; Hansen and Tjell, 1983) have found that solubility and plant uptake of Cd was lower in fine textured compared to coarse textured soils. Ziper el al. (1988) found that high charge density edge and planar sites of biotite adsorbed the most and desorbed the least amount of Cd, while interlayer sites of montmorillonite and vermiculite appeared to be least Cd-specific. Limited information exist to indicate what effect, if any, soil texture has on Cd uptake in desert soils.

A number of studies have shown Cd accumulation by crops to be influenced by Zn availability (Haghiri, 1974; Abdel-Sabour et al., 1988; Chaney et al., 1994). In one particularly relevant study, the application of Zn rates up to 5 kg/ha were found to substantially decrease Cd concentrations in wheat produced on soils prone to Zn deficiency (Oliver et al., 1994). In another study, the tendency for increased Cd uptake with increased salinity was reduced by Zn fertilization (Khoshogoftar et al., 2004). Soils in Arizona are generally marginal with respect to Zn.

Studies show that Cd uptake is enhanced by elevated levels of salinity, or specifically high soil chloride (Bingham et al., 1984; Li et al., 1994; Norvell et al., 2000; Khoshgoftarmanesh et al., 2006). It is possible that variation in the salinity of soils and irrigation waters used for spinach production in the desert southwest affect Cd accumulation in the desert.

Studies in Australia have shown high levels of cumulative P fertilization increased the Cd accumulation by crops (Williams and David, 1976). However, another study reported long-term P fertilization from experimental plots in the Midwestern and southern United States did not increase Cd levels in plants (Mortvedt, 1987). Similarly, in England, Cd uptake by crops was only increased by P fertilization in un-limed acid soils (Nicholson et al., 1994). Clearly, Cd does accumulate in agricultural land due to P fertilization but in some cases it may take up to a century or longer for Cd to reach problematic levels in the soil (De Boo, 1990; Modaihsh et al., 2001). Assuming an average P fertilization rate of 600 kg MAP/ha, Cd removal by crops was less than 5% of that applied, indicating a potential for accumulation. Generally, Cd is not leached from soil but much of the Cd added to soil with P fertilizers appears to be irreversibly fixed into non-bioavailable pools (Hamon et al., 1998). Based on preliminary evidence we collected to date, we do not believe P fertilizer is a major source of Cd to our crops. However, we wish to explore this further as an ancillary objective of our intensive sampling.

In addition to having a reliable and predictable soil test algorithm, it is important that the soil test be based on a good representative sample. The variation of metals within production fields in the desert was unknown. Knowledge of this variation is needed in developing soil sampling protocols.

The objectives of these studies was to collect high resolution samples from typical production blocks in the desert and use paired soil and spinach tissue samples to evaluate the important soil properties affecting Cd concentrations of spinach. These data are needed as a prerequisite to developing soil testing as a management tool.

Research Methods

Sampling

Paired composite soil samples were collected from over 50 spinach production units in the desert. The planting schedules were obtained from the growers. The cultivar in each planting block was recorded. Soil samples were collected before planting and tissue samples were collected immediately before harvest. The composite soil sample represented 15 to 20 individual samples collected across the production block. The composite tissue samples were also the composite of 15 to 20 sub-samples.

In addition to these paired samples, we collected high resolution soil samples from eight production blocks. These samples were based on points set by a grid or zone sampling scheme. All grid or zone sample points in each production block were GPS referenced. Each GPS referenced point in all fields represented 7 to 10 composited sub-samples over the area represented by each point. In five of these eight sites, we collected corresponding tissue samples. The tissue samples all represent a composite of 7 to 10 tissue samples over the area represented by each point.

Sample processing

All soil samples collected were placed in an empty greenhouse for drying. All tissue samples were washed with DI water to remove dust, weighed, placed in a sterile paper bag, dried in a drying oven at 65C, and weighed again. These data were used to convert all Cd data back to a fresh weight basis. Soil and tissue samples are typically ground in metal mechanical mills. However, because we were concerned about potential contamination from these metal mills, all samples were ground by hand in ceramic crucibles.

Soil Analysis

All soils were extracted for metals using the DPTA method (Amacher, 1996). The concentrations of Zn and Cd in the extracts was determined using inductively coupled plasma mass spectroscopy. Saturation percentage was used as an estimate of soil texture and was determined as described by Stiven and Khan, (1966). Soil pH was determined according to Thomas, (1996), salinity using the electrical conductance method described by Rhoades et al. (1996), and chloride using the silver titration method described by Frankenberger et al., (1996). Soil P was determined colorimetrically after sodium bicarbonate extraction (Kuo, 1996).

Plant Tissue Analysis

All plant tissue was digested by microwave digestion using nitric acid and peroxide. The Zn and Cd concentration were determined using inductively-coupled plasma mass spectroscopy. By convention, tissue concentrations used for nutritional diagnosis are reported on a dry weight (DW) basis. However, concentrations of health concern are reported on a fresh (FW) weight basis since product is consumed fresh. Therefore, we will present Zn concentrations on a dry weight basis and Cd and Pb concentrations on a fresh weight basis.

Statistical Analysis

All data were analyzed using the SAS statistical package (**SAS Institute, 2011**). Relationships between soil tests and plant concentrations were evaluated using regression analysis. Differences in the Zn fertilization studies were evaluated using the ANOVA routine. Correlations between tissue metal concentration and soil properties were evaluated using the PROC CORR routine.

Results

Current Situation with Respect to Compliance

The DPTA soil test Cd levels and corresponding spinach tissue levels across the desert production region from 2013 to 2016 are shown in Table 1. The European Union (EU) is enforcing maximum levels (MLs) of cadmium in edible tissue of leafy vegetables of 200 µg/kg FW. The FDA has not yet chosen to regulate Cd in food crops but the California Department of Public Health (CDPH) is considering an ML as low as 550 µg/kg FW. The shippers in California would mandate that all growers in the desert, including those in Arizona, comply with any CDPH ML. Of all the spinach samples we collected from 2013 through 2016, 54% would exceed the EU ML for Cd of 200 ug/kg FW but none would approach a proposed CDPH ML of 550 ug/kg FW (Tables 1 and 3 and Figures 5b and 6b). In fact, less than 2% of the spinach in the desert would exceed concentrations of 400 ug/kg FW Cd.

Many shippers are requiring growers in Arizona to only plant spinach in fields testing less than 2 mg/kg total Cd (personal communication, Yuma growers). For reasons discussed below, we focused on the DPTA soil test rather than total soil Cd. DPTA soil test Cd values are approximately 50% those total Cd levels measured after acid digestion (see Figure 1a and discussion below). The highest values we observe in the desert from DPTA extractions is 0.3 or less mg/kg, and doubling that would be well below the 2 mg/kg total soil Cd soil test threshold mandated by some shippers (Tables 1 and 2 and Figures 2b, 3b, 4b).

Cd Soil Testing

As noted above, soil testing is one possible management tool to select fields that have a lower probability of resulting in metal compliance issues. However, a pre-requisite to using soil testing is having a predictive test. In 2013-2014, with funding from the Arizona Specialty Crop

Block Grant Program, we evaluated four soil test extractants including the Mehlich I, the Mehlich III, the DPTA, the Ammonium bicarbonate DPTA, as well as total soil Cd after digestion. The MIII and AB-DPTA are multi-nutrient extractants employed by some commercial soil testing laboratories. Overall, we found the DPTA, AB-DPTA, and the total after digests equally effective for prediction of plant Cd levels. The DPTA is currently used by most commercial soil testing laboratories to make metal micronutrient fertilizer recommendations and can be utilized for Cd with minimal expense. The AB-DPTA is not used by most commercial soil testing facilities. Typically, total soil elements are poorly correlated with plant uptake because only a small pool is bioavailable, and total soil contents are rarely used as indices to predict uptake. This does not seem to be the case for Cd where the DPTA and total Cd after digestion are highly correlated (Figure 1). Overall DPTA extraction seems to give values 50% those of Cd levels measured after acid digestion of soil. Some reports in the literature suggest that the DPTA test is a better predictor of plant Cd concentrations than measurements of total Cd because it is a direct measurement of bioavailability. However, based on the limited data we have collected in our studies, both DPTA extractable and total soil Cd seem to be equally reliable. However, acid digests are more costly, result in waste disposal issues, and commercial soil testing laboratories currently use DPTA tests for micronutrient recommendations. Therefore, for logistical and economic reasons, we selected the DPTA test for further evaluation and all subsequent discussion will be based on the DPTA test.

The major purpose of the CPS funding was to evaluate spatial variation of Cd in commercial spinach fields. A summary of the data collected during 2016 are shown in Table 2 and 3. Selected data are plotted in Figures 2, 3, 4, 5, and 6. As expected, soil test variation at the field level is much lower than that across the region for Zn and Cd. The close means and medians shows that data are also generally normally distributed. This lower variation and normal distribution suggest that a good composite representative soil sample can be collected from the 5 to 10 acre spinach production blocks typically used in the region. Nevertheless, it is important that due diligence be implemented to collect good representative samples across the production block. This would involve composting about 20 soil cores collected in a zig-zag pattern across the block.

While the DPTA soil test was among the best evaluated it is far less than perfect. There is variation, suggesting some factors other than just soil bioavailable Cd affect Cd accumulation by the plants. These may include soil chemical and physical properties as well as plant genetics. These are discussed in more detail below. In order to interpret soil test Cd levels, we need to use probability for making management decisions with respect to compliance challenges. For this we need a target reference point. As noted above, no spinach in the desert seems to accumulate Cd concentrations that approach 550 mg/kg FW. Although we do not generally export spinach into EU markets we will use their ML as an example. As shown in Table 4, to have a 90% and 70% probability that our spinach would be lower than 200 mg/kg Cd FW, we would have to plant into soils that have pre-plant soil test levels of less than 0.1 and 0.125 mg/kg DPTA Cd, respectively. Even planting into soils with 0.125 to 0.15 mg/kg DPTA Cd would result in a 50% chance of producing spinach exceeding 200 ug/kg FW Cd. This limits its application as a management tool, so work was needed to identify other important soil properties affecting spinach Cd concentrations and utilize these data to improve the predictive soil test algorithm.

Cd and Zn

We need to discuss Cd soil testing and plant uptake in relationship to soil and plant zinc nutritional status because as noted above Cd plant accumulation is influenced by Zn. Probability of crop response to Zn fertilization is related DPTA extractable soil test levels. Generally, a growth and yield response is probable when soils DPTA Zn levels are less than 0.5 mg/kg. Responses are possible in the range 0.5 to 1.5 mg/kg and improbable when levels exceed 1.5 mg/kg. A review of all the data we collected in the desert as part of these studies show no sites with DPTA Zn levels <0.5 mg/kg (Tables 1 and 2 and Figures 2a, 3a, and 4a). However, 49% of the sites we sampled were in the range of 0.5 to 1.5 mg/kg where response is possible and the remainder of the sites exceeded 1.5 mg/kg where response is unlikely. A range of 50 to 70 mg/kg Zn in spinach leaves is considered adequate. Of all the sites we sampled, 13% of the spinach samples had Zn leaf concentration less than 50 mg/kg, 39% had Zn leaf concentrations in the range 50 to 75 mg/kg, and the remainder had leaf Zn concentrations exceeding 75 mg/kg (Table 3 and Figures 5a and 6a). Overall, these data indicate that spinach production fields in the desert straddle the margin between Zn adequacy and deficiency with most sites having adequate Zn for crop production.

A simple correlation of spinach Cd concentration to a range of soil properties for all paired soil and tissue samples we collected to date show spinach Cd concentrations negatively correlated to DPTA soil test Zn level (Table 6). In order to explore possible crop responses to Zn and the possibility of Zn fertilization reducing Cd tissue concentrations we conducted six Zn fertilizer experiments in 2015-2016. Three of the six studies resulted in significant yield responses to Zn fertilization, usually to rates lower than 50 kg Zn/ha (Table 5). Although Zn tissue concentration increased in these studies, high rates of Zn fertilization were not particularly effective in reducing Cd concentration to reasonable levels (Figure 7).

Other Soil Factors Affecting Spinach Cd Concentration

There were no significant relationships between spinach Cd concentration and soil pH. The range of soil pH in all the samples we evaluated ranged from 7.3 to 8.4 (Table 6). As noted above we did not expect soil pH to be a factor of prevailing importance due to the narrow range of pH values in desert soils. It should be noted that many of the studies in the literature where pH effects were reported were based on liming acid to neutral soils and all desert soils have free lime. There was no significant correlation to SP, which is an indirect measure of soil texture. Soils we evaluated ranged from loamy sand to silty clay loam. It seems other soil properties are more important in the desert than soil texture.

The negative correlation between spinach Cd concentration and soil test Zn is noted above. Spinach Cd concentrations were also negatively correlated to soil test P. Soil test P would be a reflection of P fertilization history and the MAP fertilizer used in the region contains Cd. However, the amounts of Cd applied with P fertilizer are low relative to the natural soil pool of plant available Cd. It seems the major effect of P in desert soil is to reduce Cd uptake. To what extent this is a result of P rendering Cd less soluble in the soil (Kim et al., 2015) or to antagonize the uptake of Cd by the plant, as it does with Zn, is not known.

As reported by others (Bingham et al., 1984; Li et al., 1994; Norvell et al., 2000; Khoshgoftarmanesh et al., 2006), spinach Cd concentrations were positively correlated to soil Cl concentrations. Interestingly, it was more strongly correlated to total salinity as determined by electrical conductance, which would include Cl, but also include other salts.

Overall, these data show that in addition to spinach Cd concentrations being correlated to soil test Cd, they were equally negatively and positively correlated to other soil properties routinely measured in soil testing laboratories. This indicates that as we enhance our data base we will be able to use step-wise regression to identify the most important parameters affecting spinach Cd concentrations, and compose a predictive algorithm that provides a much better prediction than soil test Cd alone.

Genetic Variation

With a study funded by the Arizona Specialty Block Grant program we obtained a collection of spinach germplasm from the Plant Introduction Center in Iowa. Evaluating this collection in a greenhouse study we found significant genetic variation in Cd concentrations. This observation is consistent with that reported by others (Grant et al., 2008; Wang et al., 2007) and shows the potential for using plant breeding as a strategy to reduce Cd exposure. However, it also potentially complicates using soil testing as a management tool. In 2014, we began tracking cultivars used in our paired samplings. The number of spinach cultivars used in the desert is large and as of this reporting we have insufficient numbers of all the cultivars utilized to determine if cultivar would significantly confound soil test interpretation. As we continue to enlarge our database we will be able to discern any meaningful differences among commercial cultivars used.

Acknowledgement

This report included data where both CPS/YCEDA and the Arizona Specialty Crop Block Grant Program funded activities and analysis. Interpretation required all the data. The CPS/YCEDA funding specifically funded high resolution soil sampling, allowed an expansion of the paired soil and tissue samplings, and allowed three additional Zn fertilizer experiments.

Literature Cited

Abd-Elfattah, A., and K. Wada. 1981. Adsorption of lead, copper, zinc, cobalt and cadmium by soils that differ in cation-exchange materials. J. Soil Sci. 32: 271-283.

Abdel-Sabour, M. F., J. J. Mortvedt, and J. J. Kelsoe. 1988. Cd-Zn interactions in plants and extractable Cd and Zn fractions in soil. Soil Sci. 145:424-431.

Adams, M. L., F. J. Zhao, S. P. McGrath, F. A. Nicholson, and B. J. Chambers. 2004. Predicting cadmium concentrations in wheat and barley grain using soil properties. J. Environ. Qual. 33:532-541.

Alloway, B.J., A. R. Tills, and H. Morgan. 1984. The speciation and availability of cadmium and lead in polluted soils. In Hemphill D.D. (ed.), Trace substances and human health. Proceedings XVIII Annual Conference, Columbia, Missouri, USA. University of Missouri, 187-201.

Amacher, M. C. 1996. Nickel, Cadmium, and Lead. In Sparks et al. (eds.). Methods of Soil Analysis. Part 3. Chemical Methods Number 5. Soil Sci, Am. Book Series. Madison, WI.

ATSDR. 1990. Toxicological profile for Cd and Pb (draft for public comment) prepared by Clement. International Corporation for US Department of Health and Human Services, Public Health, Agency for Toxic Substances and Disease Registry. 1997a.

Berg, T., and D. Licht. 2002. International legislation on trace elements as contaminants in food: A review. Food Add. Contamin. 10: 916-927.

Bingham, F. T., G. Sposito, and J. E. Strong. 1986. The effect of chloride on the availability of cadmium. J. Environ. Qual. 13:71-74.

Chaney, R. L., C. E. Green., E. Filcheva, and S. L. Brown. 1994. Effect of Fe, Mn, and Zn enriched biosolids compost on uptake of Cd by lettuce from Cd-contaminated soils. Pp. 205-207. In C. E. Clapp, W. E. Larson, and R. H. Dowdy (eds.). Sewage Sludge: Land utilization and the environment. American Society of Agronomy, Madison, WI.

Christensen, T.H. 1984a. Cadmium soil sorption at low concentrations: I. Effects of time, cadmium load, pH and calcium. Water, Air and Soil Pollution 21, 105-114.

De Boo, W. 1990. Cadmium in agriculture. Toxic. and Environ. Chem. 27:55-63.

Eriksson, J. E. 1990. A field study on factors influencing Cd levels in soils and grains of oats and winter wheat. Water Air Soil Pollut. 53:69-81.

Forbes, E.A., A. M. Posner, and J. P. Quirk. 1976. The specific adsorption of divalent Cd, Co, Cu, Pb and Zn on goethite. J. Soil Sci. 27, 154-166.

Frankernberger, W. T., M. A. Tabatabi, D. C. Adriano, and H. E. Doner. 1996. Bromine, Chlorine, and Florine. In Sparks et al., (eds). Methods of Soil Analysis. Part 3. Chemical Properties. Soil Sci. Soc. Am. 5. American Society of Agronomy, Madison, WI

Gartrell, M. J., J. C. Crum, D. C. Podrebarac and E. L. Gunderson. 1986. Pesticides, selected elements, and other chemicals in adult total diet samples, October 1980-March 1982. J. Assoc. Off. Anal. Chem. 69: 141-161.

Grant, C. A, J. M. Clarke, S. Duguid, and R. L. Chaney. 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. Sci. Total Environ.390: 301-310.

Gunderson, E. L. 1988. FDA Total Diet Study. April 1982-April 1984, dietary intakes of pesticides, selected elements, and other chemicals J. AOAC Int. 71: 1200-1209.

Gusenleitner, J., K. Aichberger, and W. Nimmervoll. 1982. Die Wirkung steigender

Cadmiumgaben auf das Wachstum von Italienischem Raygras (*Lolium multiflorum*) in Abhängigkeit von der Bodenart. Mitteilungen der Österreichischen Bodenkundlichen Gesellschaft 25: 52-74.

Haghiri, F. 1974. Plant uptake of Cd as influenced by cation exchange capacity, organic matter, Zn, and soil temperature. J. Environ. Qual. 3:180-182.

Hallenback, W. 1984. Human health effects of exposure to cadmium. Experientia. 40: 136-142.

Hamon, R. E., M. J. Mclaughlin, R. Naidu, and R. Correll. 1998. Long-tern changes in cadmium bioavailbility in soils. Environ. Sci. Technol. 32:3699-3703.

Hansen, J.A. and J. C. Tjell. 1983. Sludge application to land - Overview of the cadmium problem. In Davis, Hucker and Hermite (eds.), Environmental effects of organic and inorganic contaminants in sewage sludge. D. Reidel Publ. Co., Dordrecht, Holland, 91-113.

Khoshgoftar, A. H., H. Shariatmadari, N Karimian, M Kalbasi, S. E. A. T. M. van der Zee, and D. R. Parker. 2004. Salinity and zinc application effects on phytoavailability of cadmium and zinc. Soil Sci. Soc. Am. J. 68:1885-1889.

Khoshgoftarmanesh, A. H., H. Shariatmadari, N Karimian, M Kalbasi, and S. E. A. T. M. van der Zee. 2006. Cadmium and zinc in saline soil solutions and their concentrations in wheat. Soil Sci. Soc. Am. J. 70:582-589.

King, L.D. 1988. Effect of selected soil properties on cadmium content of tobacco. J. Environ. Qual. 17: 239-245.

Kim., S. U., V. N. Owens, Y G. Kim, S. M. Lee, H. C. Park, K. K. Kim, H. J. Son, and C. O. Hong. 2015. Effect of Phosphate Addition on Cadmium Precipitation and Adsorption in Contaminated Arable Soil with a Low Concentration of Cadmium. Environ Contam. Toxicol.95: 675-679

Kuo, S. 1996. Phopshorus. In Sparks et al. (eds.). Methods of Soil Analysis. Part 3. Chemical Methods Number 5. Soil Sci, Am. Book Series. Madison, WI.

Li, Y., R. L. Chaney, and A. A. Schneiter. 1994. Effect of soil chloride level on cadmium concentration in sunflower kernels. Plant Soil 167: 275-280

Maclean, A. J. 1976. Cadmium in different plant species and its availability in soils as influenced by organic matter and additions of lime, P, Cd, and Zn. Can. J. Soil Sci. 56:129-138.

Modaihsh, A. S., A. E. Abdallah, and M. O. Mahjoub. 2001. Accumulation of cadmium in arid soils as affected by intensive phosphorus fertilization. Arid Land Res. Manag. 15:173-181.

Mortvedt, J J. 1987. Cadmium levels in soils and plants from some long-tern soil fertility experiments in the United States of America. J. Environ. Qual. 16:137-142.

Mortvedt, J. J., D. A. Mays, and G. Osborn. 1981. Uptake by wheat of cadmium and other heavy metal contaminants in phosphate fertilizers. Soil Sci. 134.185-192.

Nicholson, F. A., K. C. Jones, and A. E. Johnston. 1994. Effect of phosphate fertilizer and atmospheric deposition on long-tern changes in the cadmium content of soils and crops. Environ. Sci. Techol. 28:2170-2175.

Norvell, W. A., J. Wu, D. G. Hopkins, and R. M. Welch. 2000. Association of cadmium in durum wheat grains with soil chloride and chelate-extractable cadmium. Soil Sci. Soc. Am. J. 64:2162-2168.

Oliver, D. P., R. Hannam, K. G. Tiller, N. S. Wilhelm, R. H. Merry, and G. D. Cozens. 1994. The effects of zinc fertilization on cadmium concentration in wheat grain. J. Environ. Qual. 23:705-711

Oliver, D. P., K. G. Tiller, M. K. Conyers, W. J. Slattery, A. M. Alston, and R. H. Merry. 1996. Effectiveness of liming to minimize uptake of cadmium by wheat and barley grain grown in the field. J. Agric. Res. 47:1181-1193.

Pennington, J. A. T., B. E. Young, D. B. Wilson, R. D. Johnson, and R. D. Vanderveen. 1986 Mineral content of food and total diets: the selected minerals in food surveys, 1982 to 1984. J. Am. Diet. Assoc. 1986, 86, 876-891.

Rhoades. J. D. 1989. Salinity, electrical conductivity and total dissolved salts. In Sparks (eds).Methods of Soil Analysis. Part 3. Chemical Properties. Soil Sci. Soc. Am. 5. AmericanSociety of Agronomy, Madison, WI.

SAS Institute Inc., SAS 9.1.3 Help and Documentation, Cary, NC: SAS Institute Inc., 2002-2004.

Sauve, S., W. A. Norvell, M. McBride, and W. Hendershot. 2000. Speciation and complexation of cadmium in extracted soil solutions. Environ. Sci. Technol. 34:291-296.

Stiven, G. A., and M. A. Khan. 1966. Saturation percentage as a measure of soil texture in the lower Indus Valley. J. Soil Sci. 17:255-263.

Street, J.J., W. L. Lindsay, and B. R. Sabey. 1977. Solubility and plant uptake of cadmium in soils amended with cadmium sewage sludge. J. Environ. Qual. 6, 72-77.

Thomas, G. W. 1996. Soil pH and Acidity. In Sparks et al. (eds.). Methods of Soil Analysis. Part 3. Chemical Methods Number 5. Soil Sci, Am. Book Series. Madison, WI.

Tiller, K.G., Nayar, V.K. & Clayton, P.M. 1979. Specific and non-specific sorption of cadmium by soil clays as influenced by zinc and calcium. Aust. J. Soil Res. 17, 17-28.

Wagner, G. J. 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron. 51:173-212.

Walker, R. and J. L. Herman. 2000. Summary and conclusions of a joint FAO/WHO expert committee on food additives. Report 55. World Health Organization: Geneva. http://www.who.int/pcs/jecfa/.

Wang, J. W. Fang, Z. Yang, J. Yuan, Y. Zhu, and H. Yu. 2007. Inter- and intraspecific variation of cadmium accumulation of 13 leafy vegetable species in a greenhouse experiment. J. Agric. Food Chem. 55, 9118-9123.

Williams, C. H., and D. J. David. 1976. The accumulation in soil of cadmium residues from phosphate fertilizers and their effect on the cadmium content of plants. Soil Sci. 121:86-93.

Ziper, C., S. Komarneni, and D. E. Baker. 1988. Specific cadmium sorption in relation to the crystal chemistry of clay minerals. Soil Sci. Soc. Am. J. 52: 49-53.

Outcomes and Accomplishments

Except for the EU ML for spinach, these data show that the soil used to produce spinach in the desert and the spinach itself, are in compliance with public and industry mandated compliance criteria. However, because soil testing would be a useful tool for managing spinach Cd levels, we conducted high resolution soil samples from typical production blocks in the desert to assess in-field variability and develop sampling protocols. We also collected paired soil and spinach tissue samples to evaluate the important soil properties affecting Cd concentrations of spinach. These data were needed as a prerequisite to developing soil testing as a management tool. The high resolution soil sampling shows generally low in-field variation and normal distribution of observed values for Cd and suggest that a good composite representative soil sample can be collected from the 3 to 10 ha spinach production blocks typically used in the region. While the DPTA soil test was among the best evaluated, it was not sufficiently predictive of spinach Cd concentrations to be utilized as a management tool alone. Data show that in addition to soil test Cd, soil test Zn, soil test P, and soil salinity were correlated to spinach Cd concentrations and need to be incorporated into any soil test algorithm used to predict spinach Cd concentrations While spinach Cd concentrations were negatively correlated to soil test Zn levels, the application of high rates of Zn fertilizer to mitigate Cd uptake was only marginally effective. However, modest rates of Zn fertilization to correct Zn nutrient deficiency appear justified.

Summary of Findings and Recommendations

It seems that except for the EU ML, nearly all the soil used for spinach production, and the spinach itself produced in the in the desert, tests below most emerging public and industry self-imposed standards for Cd. The industry in the desert should not be overburdened with excessive sampling and monitoring costs related to Cd compliance at this time. Continued research to fully develop a predictive soil test is needed now that we have identified important soil properties affecting Cd uptake. We will continue this effort with funding from a new Arizona Specialty Crop block grant that begins October 1, 2016. The development of this test will provide a short term management solution. A more effective long term solution should be aimed at developing spinach cultivars with a lower propensity for Cd accumulation as our research show genetic variation in spinach germplasm. These data show that the application of large rates of Zn to mitigate Cd uptake in desert production is inconsistent, only marginally effective, and cannot be economically justified. However, more modest rates of Zn fertilizer to correct Zn nutrient deficiencies appears justified for many desert soils.

APPENDICES

Publications and Presentations

None yet

Budget Summary (required)

Tables and Figures (Below)

Table 1. Metal concentrations of soil and spinach produced in paired soil and tissue samples collected over three growing seasons.

Season	Number	Metal	Mean	Standard Deviation of Mean	Median	Minimum	Maximum
Soil DPTA (mg/kg)							
2013-2014	25	Cd	0.11	0.04	0.10	0.04	0.19
2014-2015	45	Zn	1.89	0.79	1.57	1.09	3.82
		Cd	0.13	0.02	0.13	0.07	0.17
2016-2016	76	Zn	1.49	0.54	1.33	0.74	3.41
		Cd	0.16	0.05	0.15	0.07	0.32
Spinach Tissue Metal Concentration[1] (mg/kg DW for Zn and ug/kg FW for Cd and Pb)							
2013-2014	25	Cd	127	90.4	94.0	40.7	407.2
2014-2015	45	Zn	86	30.5	73.7	51.4	165.5
		Cd	207.8	83.0	200.0	97.3	448.9
2016-2016	76	Zn	63.4	22.9	60.7	22.3	122
		Cd	226.5	86.3	220.5	63.2	453.4

[1]Tissue concentrations used for nutritional diagnosis are reported on a dry weight (DW) basis. However, concentrations of health concern are reported on a fresh (FW) weight basis since product is consumed fresh.

Table 2. Soil test metal concentration from high resolution soil samples collected in 2016.

Site	Production Block Size (ha)	Sample Resolution (ha)	Metal	Mean	Standard Deviation of Mean	Median	Minimum Value	Maximum Value
				Soil DPTA (mg/kg)				
Coachella Valley, Riverside Co, CA	1.1	0.07	Zn	5.0	1.1	4.6	4.1	8.6
			Cd	0.11	0.01	0.11	0.10	0.13
Bard Valley #1, Imperial Co., CA	0.5	0.02	Zn	2.3	0.24	2.3	1.8	3.1
			Cd	0.17	0.01	0.17	0.14	0.22
Bard Valley #2, Imperial Co., CA	3.1	0.15	Zn	1.74	0.25	1.74	1.25	2.44
			Cd	0.21	0.02	0.20	0.17	0.28
Bard Valley #3, Imperial Co., CA	1.6	0.05	Zn	1.78	0.40	1.62	1.32	2.98
			Cd	0.20	0.02	0.20	0.18	0.28
Bard Valley #4, Imperial Co., CA	1.3	0.04	Zn	0.98	0.17	0.97	0.72	1.65
			Cd	0.13	0.01	0.12	0.11	0.16
Yuma Valley #1, Yuma Co., AZ	2.5	0.125	Zn	1.30	0.13	1.27	1.06	1.60
			Cd	0.15	0.010	0.15	0.13	0.20
Yuma Valley #2, Yuma Co., AZ	2.6	0.16	Zn	1.10	0.08	1.09	0.98	1.23
			Cd	0.14	0.01	0.14	0.12	0.15
Yuma Valley #3, Yuma Co., AZ	1.2	0.05	Zn	1.52	0.73	1.31	0.89	3.71
			Cd	0.17	0.05	0.15	0.11	0.31

Table 3. Spinach metal concentrations from high resolution spinach tissues samples collected in 2016.

Site	Production Block Size (ha)	Sample Resolution (ha)	Metal	Mean	Standard Deviation of Mean	Median	Minimum Value	Maximum Value
Spinach Tissue Metal Concentration[1] (mg/kg DW for Zn and ug/kg FW for Cd and Pb)								
Coachella Valley, Riverside Co, CA	1.1	0.07	Zn	135.0	7.8	134.0	122.6	147.0
			Cd	82.4	14.3	79.8	63.4	127.0
Bard Valley #3, Imperial Co., CA	1.6	0.05	Zn	85.6	8.9	85.8	67.9	102.7
			Cd	297.6	30.9	293.3	224.9	359.5
Bard Valley #4, Imperial Co., CA	1.3	0.04	Zn	86.6	19.8	83.7	62.5	116.6
			Cd	136.8	34.3	130.1	58.8	253.1
Yuma Valley #2, Yuma Co., AZ	2.6	0.16	Zn	59.2	11.2	56.1	51.8	98.2
			Cd	241.9	28.3	244.7	200.1	287.5
Yuma Valley #3, Yuma Co., AZ	1.2	0.05	Zn	68.4	34.0	91.6	20.5	106.7
			Cd	197.5	91.7	243.4	71.1	311.6

[1]Tissue concentrations used for nutritional diagnosis are reported on a dry weight (DW) basis. However, concentrations of health concern are reported on a fresh (FW) weight basis since product is consumed fresh.

Table 4. Relationship between DPTA soil-test Cd and predicted tissue concentration.

Soil Test Cd (mg/kg)	Predicted Mean Tissue Cd mg/kg FW	Probability Tissue< 200 mg/kg FW (%)
<0.1	136	90
0.1 – 0.125	152	70
0.125-0.150	193	50
0.150-0.175	239	29
0.175-0.200	243	27
>0.200	237	13

Table 5. Response of spinach to Zn fertilization in six field experiments conducted in the desert during 2015-2016.

	Experiment					
Zn Rate (kg/ha)	1	2	3	4	5	6
0	19.4	26.4	6.2	12.8	13.4	7.6
25	21.1	32.0	6.3	13.5	13.8	8.8
50	20.8	35.1	6.4	13.4	13.8	10.0
100	21.6	37.1	7.0	14.2	14.7	9.9
200	22.5	41.4	6.7	13.7	12.8	8.5
400	20.7	39.5	6.7	14.4	12.8	10.8
	L*Q*	L**Q**	NS	NS	NS	L*

*,**. Significance at the 5 and 10% levels, respectively. Not Significant (NS) =P>0.05.

Table 6. Correlation of spinach Cd and Zn concentrations to other tissue concentrations and soil properties.

Parameter	Response	Tissue Cd
Soil Test Zn	Correlation Coefficient	-0.37
	Significance	<0.01
Soil Test Cd	Correlation Coefficient	0.37
	Significance	<0.01
Tissue Zn	Correlation Coefficient	0.02
	Significance	NS
Tissue Cd	Correlation Coefficient	
	Significance	
Soil pH	Correlation Coefficient	.01
	Significance	NS
Soil Saturation Percentage (SP)	Correlation Coefficient	0.08
	Significance	NS
Soil EC	Correlation Coefficient	0.32
	Significance	<0.01
Soil Cl	Correlation Coefficient	0.17
	Significance	0.02
Soil Test P	Correlation Coefficient	-0.33
	Significance	<0.01

Significance to 0.05 reported. Not significant (NS) of P>0.05. These correlations are based on 205 paired comparisons.

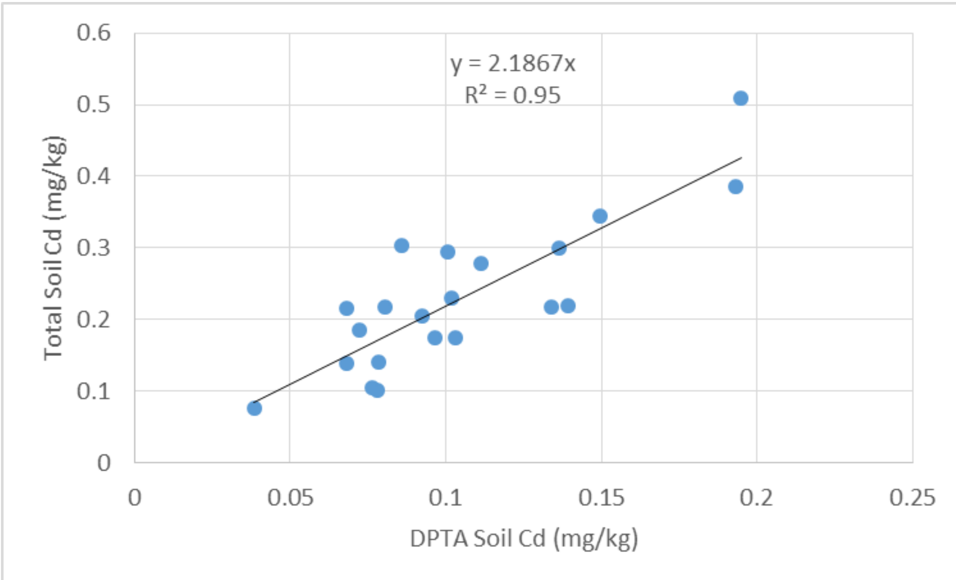
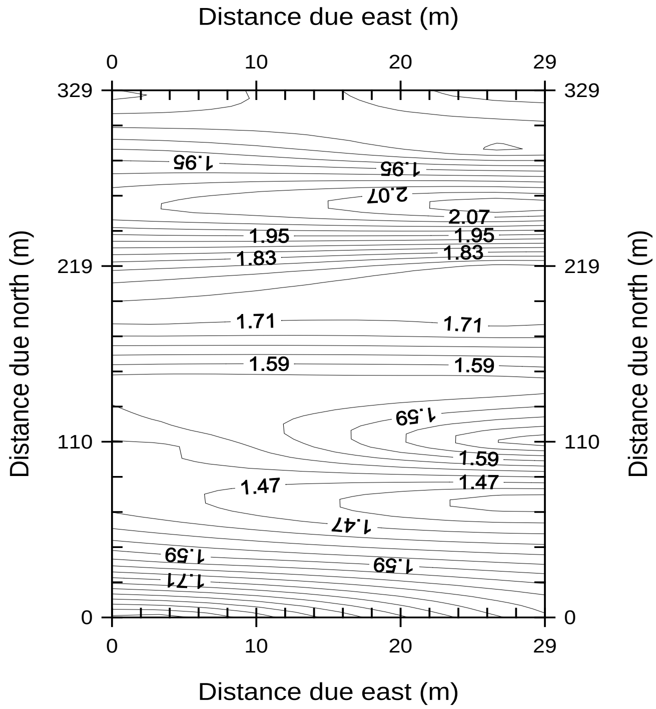
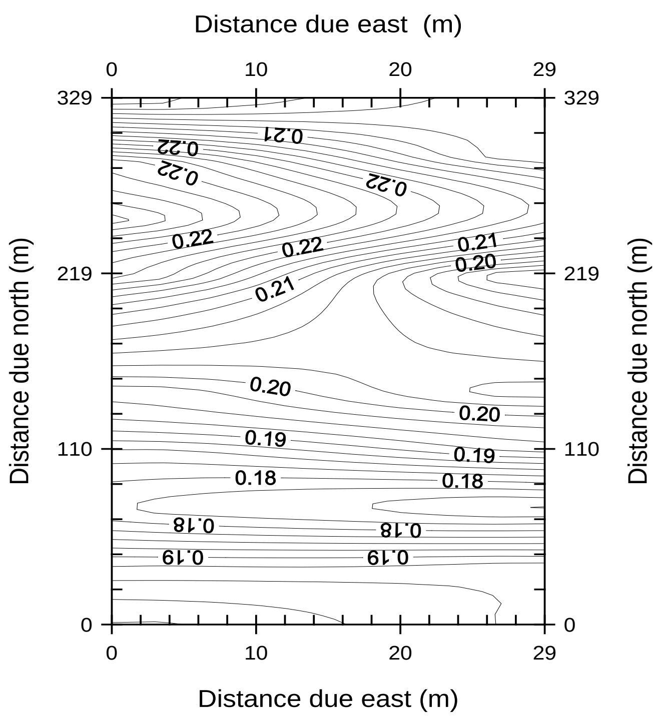


Figure 1. Relationship between (a) DPTA Soil Cd and Total Soil Cd and.

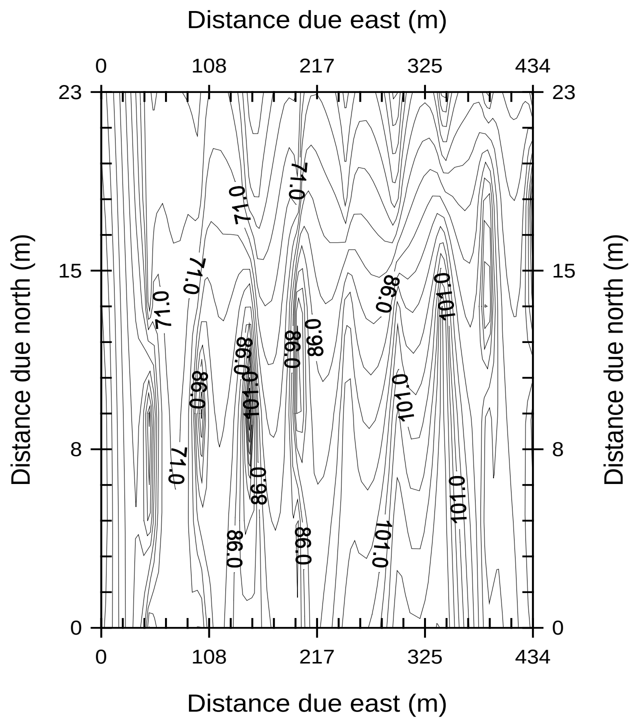


(a)

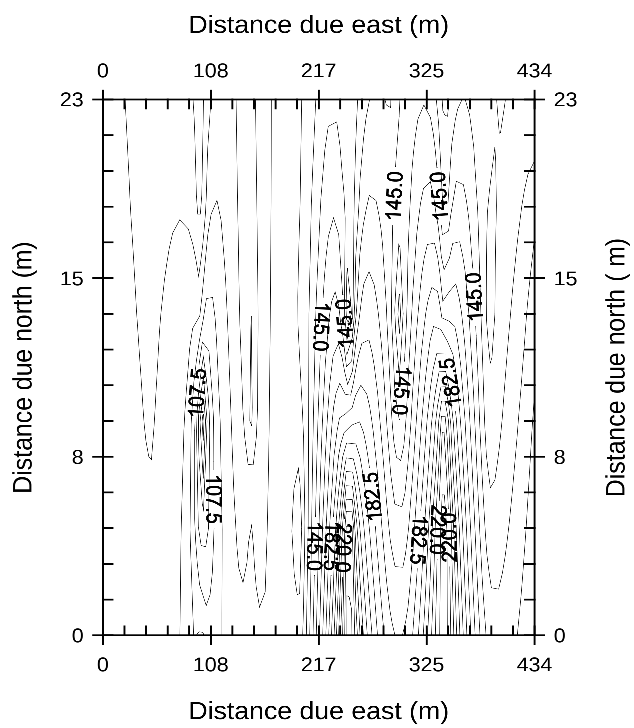


(b)

Figure 2. Soil test DPTA Zn (a) and Cd (b) in Yuma #1.

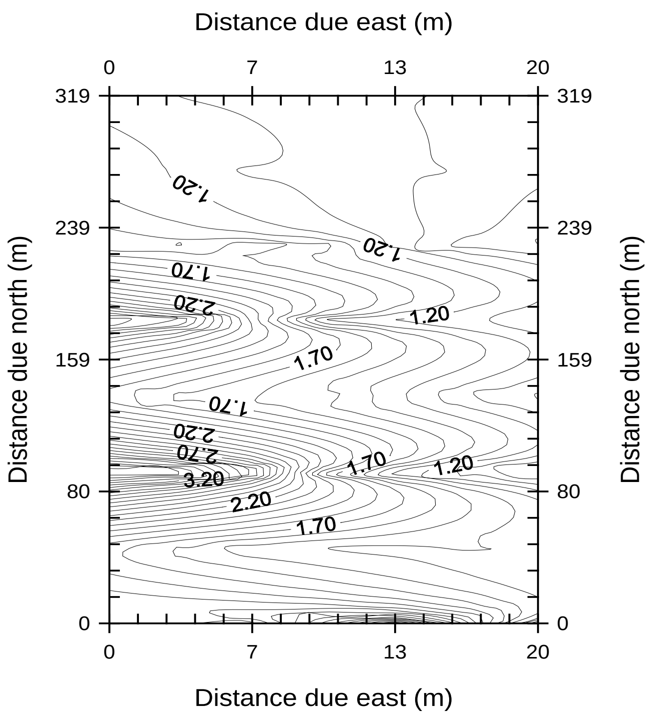


(a)

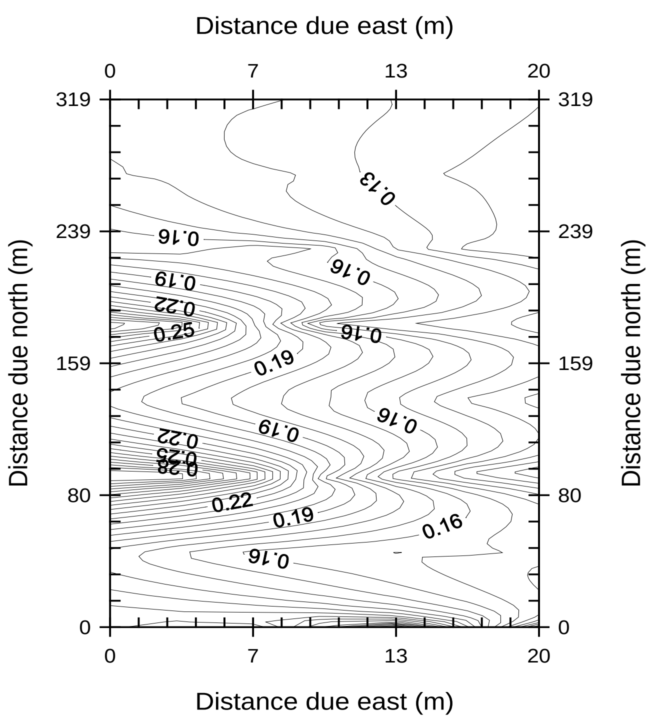


(b)

Figure 3. Soil test DPTA Zn (a) and Cd (b) for Bard#4.

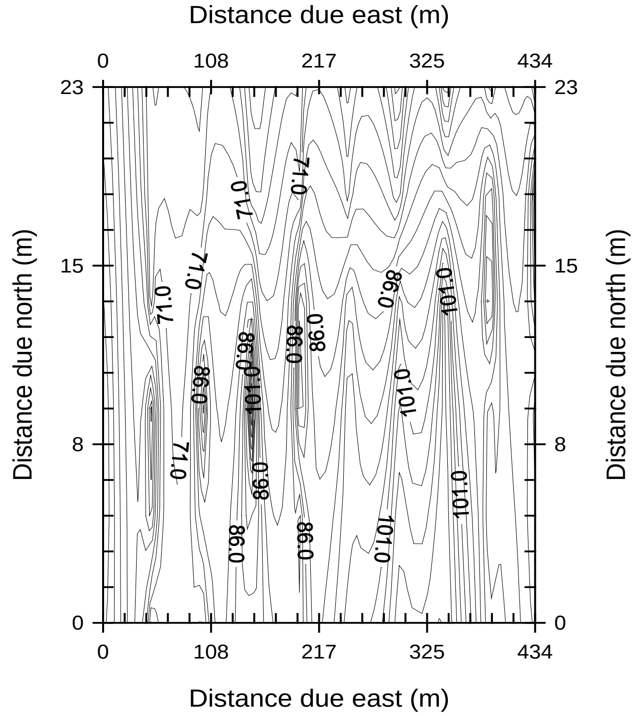


(a)

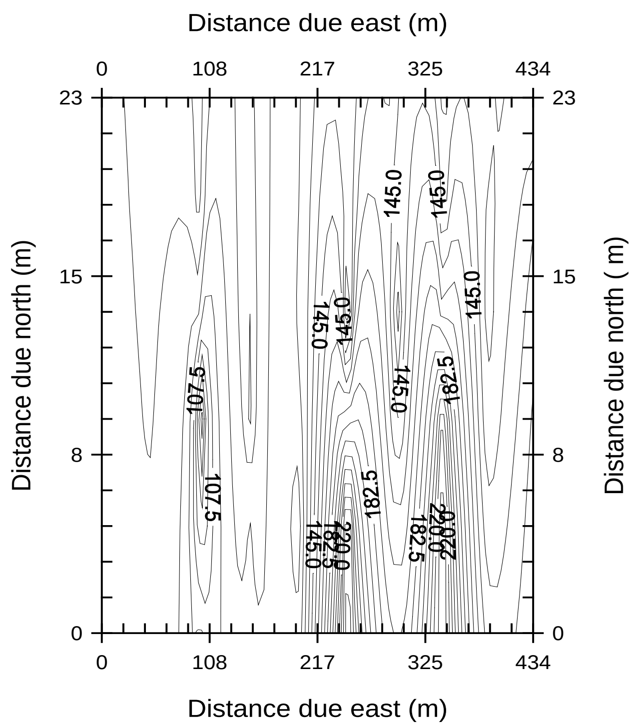


(b)

Figure 4. Soil test Zn (a) and Cd (b) for Yuma site #3.

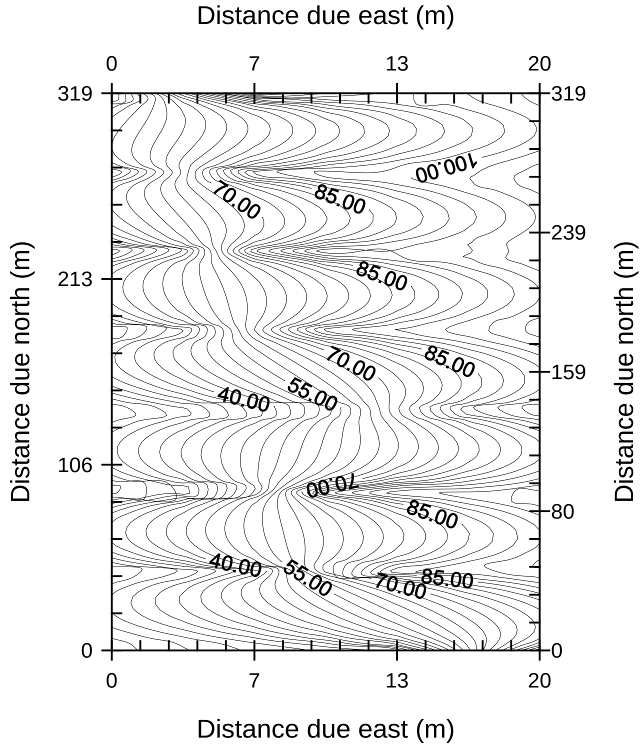


(a)

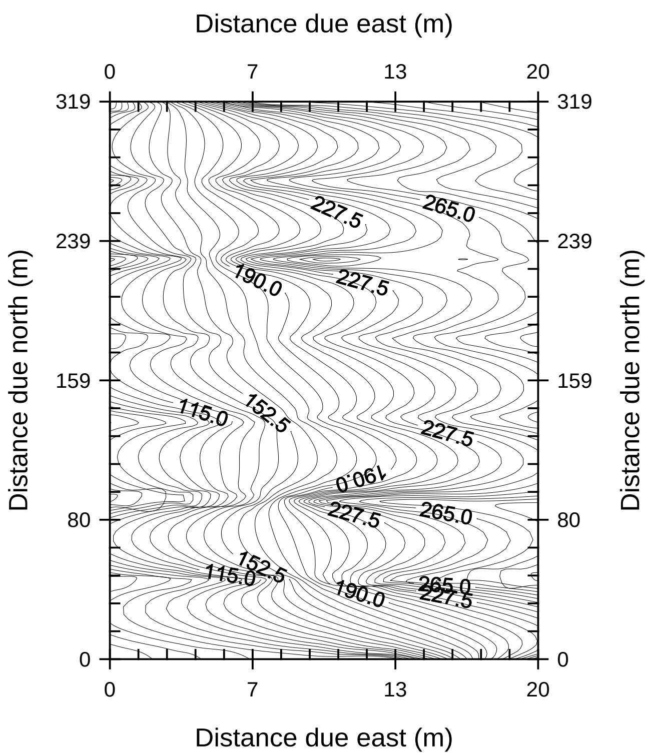


(b)

Figure 5. Spinach tissue Zn (a) and Cd (b) for Bard site #4.



(a)



(b)

Figure 6. Spinach tissue Zn (a) and Cd (b) for Yuma site #3.

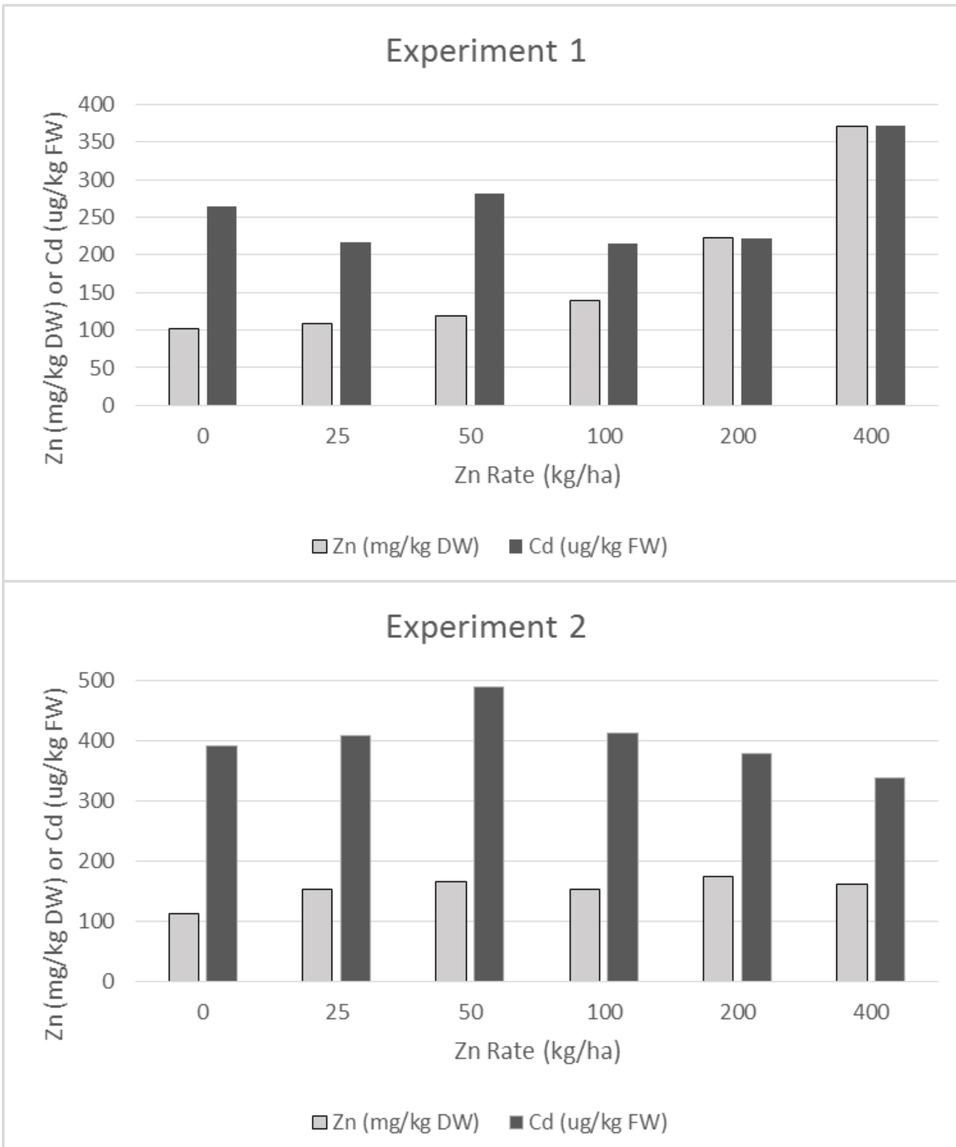


Figure 7. Spinach tissue Zn (mg/kg DW) and Cd (ug/kg FW) to Zn fertilization in two experiments. In both experiments tissue Zn increased to Zn fertilization. In experiment 1, tissue cadmium increased and in experiment two the effect was quadratic (increased then decreased).