CA Leafy Greens Research Board
April 1, 2014 – March 31, 2015
Title: Project title: Evaluation of Practices to Reduce Cadmium Uptake by Leafy Greens
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ABSTRACT:

A series of greenhouse, lath house and field trials were conducted to evaluate the relationships among soil characteristics, soil amendment strategies and crop cadmium (Cd) concentrations, with the goal of determining effective methods to minimize crop Cd uptake, particularly by spinach. Total soil Cd concentration was the most effective soil test predictor of spinach Cd concentration. Application of zinc (Zn) fertilizers to soil was the most effective measure to reduce crop Cd uptake; per unit of Zn applied, Zn chelate was more effective than zinc sulfate, which was more effective than zinc oxide. The effects of soil zinc application diminish over time, but significant reduction in crop Cd uptake was observed over multiple cropping cycles. There is a diminishing return with increasing soil Zn amendment rate, with the greatest reduction in crop Cd uptake observed with the initial 25 PPM Zn applied. The depth of Zn incorporation in soil is important, with deeper incorporation giving the greatest reduction in crop Cd uptake. Common incorporation methods (disking or mulching) provide limited effective incorporation depth. Soil application of lime, compost and biochar have given inconsistent results, with a reduction in crop Cd concentration observed in some trials and some soils, but not in others. Foliar Zn application was ineffective in reducing crop Cd uptake. High chloride content in irrigation water increased crop Cd uptake.

OBJECTIVES:

1. Conduct strip trials with Zn fertilizer and compost in commercial production fields on spinach and romaine, assessing the longevity of treatment effectiveness.
2. Evaluate the impact of foliar applications of Zn to spinach on plant Cd uptake.
3. Evaluate the impact of chloride in irrigation water on the uptake of Cd by spinach.
4. Evaluate the impact of sources of Zn on Cd uptake of spinach.
5. Evaluate alternative materials to reduce Cd uptake by spinach.
6. Within the range of concentrations likely to be able to be remediated economically, evaluate which soil characteristics are predictive of spinach Cd uptake.

PROCEDURES:

Pot trials:

A series of pot experiments were conducted using Salinas Valley soils to evaluate the effect of zinc fertilizers and soil amendments on cadmium and zinc uptake of spinach. Soils were air-dried, sieved and thoroughly blended for uniformity before use. Soil availability of Cd and Zn were evaluated both by the traditional DTPA extraction and by nitric acid digestion (‘total’ soil Cd and Zn). Table 1 lists relevant soil characteristics for trials 1-5. Experiment 1 was conducted in a greenhouse at UCD during the winter, 2014, and experiments 2-5 were conducted in a lath house at the UCCE facility in Salinas in summer, 2014. The pots used were 8” tall, 2 L volume (experiment 1), or 12” tall, 4 L volume (all other trials). All trials utilized the spinach variety ‘Tambourine’. Fertility was provided by watering with a complete nutrient solution (experiment 1), or incorporating controlled release fertilizer into pots (all other trials).

Experimental design was a randomized complete block in all trials, with either 3 or 4 replications per treatment, depending on the trial. At harvest plants were cut at the soil line, rinsed to remove adhering soil, oven-dried, ground, and analyzed for total Cd and Zn concentration.

Experiment 1

The relative efficacy of zinc amendments on spinach Cd and Zn uptake was compared. Soils were thoroughly blended with either zinc sulfate ($ZnSO_4$, 36% Zn), zinc oxide (ZnO, 72% Zn) or Zn chelate (9% Zn) at the rates specified in Table 1. Additional treatments evaluated were 'biochar' (pyrolyzed organic material, added at a rate of 0.5% of dry soil weight) and gypsum (added at an equivalent rate of 0.3% of dry soil weight). Biochar is reputed to sequester heavy metals in soil, and gypsum (a soluble Ca source) could in theory be useful in removing Cd from cation exchange sites and allowing it to leach. Pots were seeded on 18 Dec., 2013, with 5 replicate pots per soil/treatment combination. Due to poor germination, the pots were replanted with 3 week old spinach transplants on 17 Jan., 2014, 4 plants per pot. On 10 Feb. all plants were harvested, oven-dried, ground and analyzed for total Cd and Zn concentration.

Experiment 2

Three soils were used, varying in total Cd from 1.8-4.7 PPM (Table 1). Three soil treatments were compared: soil amended with $ZnSO_4$ at 80 PPM Zn filled the whole pot, soil in only the top half of pots (0-6") amended with $ZnSO_4$ at 80 PPM Zn, and unamended control soil (no Zn added). There were 4 replicate pots per soil/treatment combination. Pots were seeded on 1 May and harvested 9 June.

Experiment 3

The effect of soil properties on spinach Cd concentration was evaluated using 12 Salinas Valley soils selected to represent a wide range of Cd content (Table 1). There were 3 replicate pots of each soil. Pots were seeded on 26 June, and harvested 5 Aug. Extensive laboratory analysis was used to characterize the physiochemical characteristics of these soils, including a Cd and Zn bioavailability assay in which cation exchange resin packets (Unibest International, Walla Walla, WA) were incubated with moist soil for 3 days, then eluted with 2M HCl to measure the amount of Cd and Zn adhered to the resin beads.

Experiment 4

To test the residual effect of Zn amendment on subsequent spinach crops, the soil from experiment 2 was air-dried, rescreened potted and replanted. Unamended soil and soil amended with 80 PPM Zn (from $ZnSO_4$) were compared. A third treatment, 80 PPM Zn-amended soil only in the top 4" of the pot, was also included. There were three replicate pots per soil/treatment combination. Pots were seeded 3 July and harvested 11 Aug.

Experiment 5

To confirm the results of experiment 1 using larger pots and in an environment more typical of field conditions, the effects of Zn source and concentration were evaluated using a Lockwood soil with 3.2 PPM total soil Cd. Treatments included Zn amendment at 25 and 50 PPM from both $ZnSO_4$ and ZnO. The effect of liming was evaluated by including treatments in which control (no Zn) soil, and soil amended with 50 PPPM Zn from $ZnSO_4$, were blended with hydrated lime at a $CaCO_3$ equivalent rate of 0.1% of soil dry weight. Additionally, the depth of

Zn incorporation was also evaluated. Three treatments were compared, with the same amount of Zn applied in each pot (50 PPM Zn on a whole pot basis, from $ZnSO_4$), but distributed either in the top 3" of the pot, the top 6" of the pot, or throughout the pot. There were 4 replicate pots per treatment. Pots were seeded 12 Aug., and harvested 16 Sept.

Two additional pot trials were conducted in a greenhouse in Salinas during winter/spring, 2014.Two soils with differing levels of cadmium were used (Table 2). Soil was screened (1/8" screen) and was placed into pots (3" square by 8" deep). Each pot was fertilized with 0.1 gram potassium sulfate and 0.2 gram of 11-52-0.

Experiment 6

Twelve to sixteen seeds of the cultivar 'Tambourine' were planted on January 14th and replanted as necessary on January 21st (due to damping off). Pots were thinned to 4-6 plants per pot on February 3. Starting 10 days after seeding, pots were fertilized with 170 ppm N weekly. Foliar zinc applications were made on February 3 (1-2 true leaf) and February 10 (2-4 true leaf). Pots of each treatment were arranged in an area 10' x 10' area and the zinc treatments were applied in the equivalent of 46 GPA. The material was applied with two passes of a one tip wand with an 8008EVS nozzle. The pots were watered by delivering the water to the soil surface with a funnel to avoid washing the material from the leaves. All treatments were replicated 6 times in a randomized complete block design. Spinach plants were harvested on February 18; plants in each pot were clipped at the soil surface, weighed, triple washed (twice in tap water before a final rinse in distilled water), and dried at 150 F. Dried plant tissue samples were analyzed for total Zn and Cd concentration.

A second trial was conducted in a commercial spinach field west of Salinas on a Salinas loam soil. The trial was planted on March 17 with the variety 'Silver Whale'. Treatments were applied on April 9 (2 true leaves) and April 14 (4 true leaves) with two passes of a one tip wand with an 8008EVS nozzle applying the equivalent of 46 GPA of water. The field was sprinkler irrigated which may have washed some of the material off of the leaves during irrigation events. The harvestable portion of the plant was harvested on April 22.

Experiment 7

Twelve seeds of spinach cultivar "Silver whale" were sown in each pot, and then thinned to 4 plants/pot after establishment. Plants were irrigated with DI water adjusted to one of four levels of chloride using calcium chloride. Plants were irrigated 2-3 times per week by applying sufficient water to leach a portion through the pot. Treatments compared four levels of chloride concentration in the irrigation water (0, 50, 100 and 200 PPM). Chloride treatments were replicated 6 times in a randomized complete block design (24 pots/soil type). Plants were fertilized with nitrogen and phosphorus at planting and again with nitrogen after 2 weeks. Plants were grown for 5 weeks in the first trial (planted 1/14/14 and harvested 2/18/14) and for 7 weeks in the second trial (planted 3/12/14 and harvested 4/24/14). Growth was initially very slow during the second trial due to placement in a cooler growing house. After 4 weeks (4/7/14), plants were moved to a warmer greenhouse and grew more rapidly.

At harvest, the above ground biomass was collected, washed in DI water, dried and analyzed for Cd concentration. After harvest, soil from all reps of a given treatment and soil type was mixed together and a subsample was sent to the UC Davis Analytical Lab for a salt profile analysis, including Cl, Cd and Ca.

Field trials:

Longevity of effectiveness of soil Zn treatment

A large-scale field trial was initiated in at field with 2.35 mg Cd/kg. The objective was to establish a site that we can return to over the next few years and observe the long-term effectiveness of a zinc application to the soil. In early May the field was divided into four sections and the following materials were applied to one of the sections: 1) lime at the rate of 3 tons/A; 2) compost at 10 tons/A; 3) compost at 10 tons/A + lime at 3 tons/A; and 4) unamended control. The materials were applied with a commercial applicator.

Within each of these four field sections zinc was applied at the rate of 100 and 200 lbs Zn/A (277 and 555 lbs zinc sulfate) in strips eight 80-inch beds wide by the length of the field (the trial also included the remainder of the field with no zinc strips). The zinc sulfate was dissolved in water and sprayed on the soil surface, followed by disking. Spinach was planted on May 16 and was harvested on June 16; the first crop planted on these strips was spinach which was planted in strips and there was no opportunity to include an untreated control in this evaluation. Following the spinach crop head lettuce was planted in this field August 2, and harvested October 2.

Another large-scale study site established in 2013 was also followed over subsequent cropping cycles in 2014, but the plots were narrower and it is unclear how effectively they will hold up to movement of soil between plots by tillage. Soil samples were collected on October 1, 2013, February 19 and June 26, 2014 to evaluate the residual levels of zinc in each of the treatments.

Effect of spinach growth stage on Cd concentration

Two commercial spinach fields with total soil Cd concentration of approximately 0.3 mg Cd/kg were used to evaluate the effect of crop growth stage on spinach Cd concentration. Spinach was harvested multiple times over a 2 week period to track tissue Cd concentration from 1-2 leaf stage to ‘freezer’ stage.

Comparison of zinc sources

A trial was established on a site mapped as Salinas loam with a total soil cadmium level of 1.0 mg Cd/kg. Materials were applied to 80 inch beds and each plot was one bed wide by 15 feet long. The experimental design was randomized complete block design with three replications. The material was applied by hand to the bed tops and mulched into the soil on March 12, 2014. The trial area was planted on March 17 with the variety ‘Silver Whale’, and was harvested on April 22.

A second trial was conducted on a site with 2.0 mg total Cd/kg. The trial was a non-replicated strip comparison conducted in collaboration with Wilbur Ellis Co. All treatments were applied with commercial equipment. Powdered zinc sulfate is difficult to apply commercially because it is dusty and easily blown away from the intended application site. To solve this problem, the material was dissolved in water and sprayed onto the bed top. Solubilized zinc sulfate was compared with zinc chelate solution (also sprayed on the bed top) and granular zinc sulfate applied to the bed top. All materials were mulched into the beds. Spinach was planted on March 7 and harvested on April 8.

Effect of zinc incorporation depth

To evaluate the effective depth of Zn incorporation with common tillage approaches, soil sampling was conducted in nine commercial fields. In these fields Zn was either disked or mulched into the soil. Soil samples were then collected at three inch intervals to one foot depth.

RESULTS:

Pot trials:

Experiment 1

All zinc materials suppressed spinach Cd uptake as well as stimulated Zn uptake, radically changing the Zn:Cd ratio (Table 3). The Zn:Cd ratio has human health significance because elevated plant Zn concentration reduces Cd absorption in the human digestive system. Per unit of elemental Zn applied, zinc chelate was generally more effective than $ZnSO_4$, which was more effective than ZnO; differences in solubility, and susceptibility to precipitation out of soil solution, undoubtedly accounted for these differences.The response to gypsum and biochar was different in the two soils, with a modest reduction in Cd uptake observed in the soil 2, but no reduction in soil 1.

There was a diminishing return on Zn amendment, with the greatest % reduction in leaf Cd observed with the first 25 PPM Zn, and smaller declines with additional Zn (Fig. 1). Averaged across the soils, 25 PPM Zn application reduced leaf Cd by 55%, while 100 PPM Zn reduced leaf Cd by 75%.

Experiment 2

Amending soil with 80 PPM Zn using $ZnSO_4$ reduced spinach leaf Cd concentration by 60-65% across soils (Table 4). In pots in which only the top half (0-6”) of soil was amended with Zn, leaf Cd reduction averaged only 25%, indicating that maximum effectiveness will require the entire effective root zone to be amended. Zn amendment substantially increased spinach Zn uptake, which resulted in a dramatic increase in tissue Zn:Cd ratio.

Experiment 3

The 12 soils evaluated in this experiment varied widely in all soil properties measured (Table 5). Soil pH, cation exchange capacity (CEC) and organic matter were not correlated with spinach dry tissue Cd concentration. All measures of soil Cd were strongly correlated with spinach tissue Cd, with total soil Cd the best predictor of spinach Cd concentration. No measure of soil Zn was predictive of spinach Cd concentration; both soil Zn:Cd ratios were correlated, but they did not improve upon the relationship between soil Cd and spinach tissue Cd.

Experiment 4

This experiment demonstrated that Zn amendment of soil can suppress spinach Cd uptake for more than one cropping cycle. The soils from experiment 2, without any additional Zn application, were again planted to spinach, and the degree of reduction in spinach tissue Cd concentration was similar to that observed in experiment 2 (Table 6). This experiment also confirmed that maximum suppression of spinach Cd uptake requires amending the entire root zone. How long this suppressive effect will last in the field is not clear. While plant uptake of Zn is an insignificant factor (at a soil amendment rate of 80 PPM Zn crop Zn uptake would be< 1% of applied Zn per cropping season), soil applied Zn may undergo chemical changes over time, forming compounds of limited plant availability. Soil analysis of these soils after each cropping season showed that plant-available Zn (as estimated by DTPA extraction) was stable in the control (no Zn application) soil (Table 7). However, DTPA Zn in the Zn-amended soil declined between crop 1 and crop 2 much more than plant Zn uptake would account for, suggesting that chemical changes were indeed occurring. Additional trials are being conducted to document the effective duration of activity from a one-time Zn application.

Experiment 5

Although both zinc sources suppressed spinach dry tissue Cd concentration, $ZnSO_4$ was more effective than ZnO per unit of elemental zinc applied (Table 8). As in experiment 1, there was a diminishing return on Zn application rate; across Zn fertilizers, spinach Cd was reduced by 46% at 25 PPM Zn, and by 63% at 50 PPM Zn. Liming increased soil pH from 6.9 to 7.5, but had no significant effect on spinach Cd or Zn concentration. The importance of amending the entire root zone was again demonstrated. Treatments 4, 8 and 9 all received the same amount of Zn per pot, the treatments differing only in how deep the Zn was distributed in the pots. The greatest reduction in spinach Cd uptake occurred where the entire root zone was Zn-enriched.

Experiment 6

None of the foliar Zn treatments reduced the concentration of cadmium in spinach tissue in the greenhouse or field trials (Table 9). Foliar application 1.0 lb zinc/A as zinc sulfate increased the level of cadmium in the commercial field trial but not in the greenhouse trial. Foliar zinc as zinc chelate increased the concentration of cadmium in spinach tissue in both trials; the mechanism by which this occurred was not apparent. Foliar zinc at the rates used in these trials increased Zn tissue concentrations by 272 to 678% over the levels in the untreated control. We observed small necrotic spots on Zn-treated plants in the greenhouse trial, but not in the field trial. Foliar applications of zinc were ineffective in reducing cadmium uptake, and in some cases actually increased tissue Cd. Therefore, foliar applications of zinc are not recommended.

Experiment 7

Across the two soils used, Cd concentration in spinach increased with increasing chloride content in the irrigation water (Table 10). The effect was most pronounced at the 200 PPM Cl level, which was equivalent to approximately 6 meq/liter Cl. For irrigation water < 3 meq/liter the effect appeared to be relatively minor.

Field trials:

Longevity of effectiveness of soil Zn treatment

Both compost and lime appeared to reduce Cd uptake in spinach tissue over the unamended soil in the 100 and 200 lbs Zn/A treatments (Table 11). Lime only marginally increased soil pH in the 100 and 200 lbs Zn/A treatments. We did not detect an effect on soil organic matter in the compost amended treatments. Since no control area (no Zn or amendments) was planted in the spinach trial it was impossible to directly measure the effect of Zn treatment; however, across amendments the 200 lb/A Zn rate reduced tissue Cd concentration approximately 25% compared to the 100 lb/A Zn rate.

In the head lettuce crop planted after the spinach harvest, the Zn application made before the spinach crop decreased tissue Cd by approximately 30% (Table 12). The effect of the lime and compost application persisted as well, reducing tissue Cd beyond the level of the Zn application alone. The effect of the original Zn application was still apparent in soil as well, with

DTPA Zn levels much higher than the unamended soil. Soil DTPA Zn declined between the first and second crops after Zn application by about 25% (much more than accounted for by plant Zn uptake), again suggesting that long-term chemical changes were occurring.

In the long-term trial established in 2013, soil DTPA Zn declined over time. By October, 2014 (after 3 crops), DTPA Zn declined about 80% across Zn treatments (Table 13). Since in this field individual plots were relatively small, this sharp decline may have been partly due to mixing of soil during tillage. Despite the declining DTPA soil Zn, the level of cadmium in all three crops grown following the initial application of zinc declined. However, the degree of tissue Cd decline was dependent on the form and rate of zinc added to the soil.

Effect of spinach growth stage on Cd concentration

Across a two-week harvest window (from 1-2 leaf to ‘freezer’ age spinach) growth stage did not substantively affect tissue Cd concentration in either of two commercial fields (Table 14).

Comparison of zinc sources

There were no significant differences among treatments in spinach Cd concentration in the replicated field trial (Table 15). However, there was a trend indicating that zinc sulfate powder had lower tissue Cd than zinc sulfate granules and zinc oxide powder at the 100 and 200 lbs Zn/A rates. The addition of calcium carbonate did not appear to affect Cd uptake at this site. In the field strip (non-replicated) trial, spinach Cd concentration was most greatly reduced by zinc chelate, followed by zinc sulfate solution and then by zinc sulfate granules (Table 16). Presumably, zinc sulfate granules did not disperse as efficiency through the soil as granular material.

Effect of zinc incorporation depth

Across nine commercial fields, soil sampling revealed that most of the applied Zn remained in the top 3” of soil following incorporation, with virtually no Zn moved below 6” depth. The mean DTPA extractable soil Zn concentration across fields was 77, 28, 3 and 3 PPM in the 0-3”, 3-6”, 6-9” and 9-12” soil depths, respectively. There was a trend toward deeper incorporation with disking as compared with mulching.

Table 1. Characteristics of the soils used in the pot experiments.

					PPM Cd		PPM Zn	
Experiment	Soil	Soil series	pH	Texture	DTPA	total	DTPA	total
1	1	Lockwood	7.3	sandy loam	0.8	2.1	2.4	76
	2	Greenwood	7.3	loam	0.4	1.3	1.3	37
2	1	Lockwood	7.0	sandy loam	0.9	2.5	3.7	74
	2	Lockwood	7.3	clay loam	1.9	4.7	3.8	85
	3	Greenwood	7.3	loam	0.8	1.8	2.8	39
3	1	Lockwood	6.7	sandy loam	0.8	2.5	3.0	79
	2	Greenwood	6.9	loam	0.8	1.8	2.8	35
	3	Lockwood	7.5	clay loam	1.7	4.8	3.0	92
	4	Antioch	7.3	loam	0.3	0.5	4.4	48
	5	Salinas	6.4	clay loam	0.4	0.8	2.0	84
	6	Mocho	7.1	clay loam	0.4	1.0	1.8	93
	7	Pacheco	6.2	loam	0.4	0.9	2.3	75
	8	Salinas	7.5	loam	0.5	1.0	2.5	102
	9	Elder	5.8	sandy loam	0.8	1.7	11.0	98
	10	Lockwood	7.0	loam	1.9	5.4	2.9	81
	11	Elder	7.6	loam	1.4	3.8	3.9	104
	12	Rincon	7.5	clay loam	0.8	2.8	1.0	82
4	1	Lockwood	6.1	sandy loam	0.9	2.5	3.0	74
	2	Lockwood	6.7	clay loam	1.7	4.6	2.8	89
	3	Greenwood	6.6	loam	0.8	1.8	2.7	39
5	1	Lockwood	6.9	sandy loam	1.1	3.2	3.7	90

Table 2. Characteristics of the two soils used in the Salinas greenhouse pot trials (experiments 6 and 7).

Soil	pH	% Organic matter	EC dS/m	Ca meq/L	Mg meq/L	Na meq/L	Cl meq/L	B PPM	DTPA Zn mg/kg	Cd total mg/kg
1	7.17	1.95	3.37	22.0	7.99	6.42	10.6	0.14	2.0	1.6
2	7.41	1.02	5.01	21.6	12.47	20.83	15.6	1.12	2.2	2.3

Table 3.Effect of zinc, biochar and gypsum amendment of soil on spinach dry tissue Cd and Zn concentration, pot experiment 1.

		Spinach tissue concentration (PPM)		
Soil	Treatment	Cd[x]	Zn	Zn/Cd ratio[x]
1	25 PPM Zn from $ZnSO_4$	5.1 a	151 bc	30 d
	50 PPM Zn from $ZnSO_4$	3.5 a	164 ab	48 b
	100 PPM Zn from $ZnSO_4$	3.6 a	223 a	64 a
	50 PPM Zn from ZnO	6.3 a	144 bc	23 e
	100 PPM Zn from ZnO	4.2 a	160 b	38 bc
	25 PPM from Zn chelate	4.2 a	134 bcd	32 cd
	Gypsum at 0.3% by weight	16.5 c	99 cde	6 f
	Biochar at 0.5% by weight	12.2 b	63 e	5 f
	Unamended soil	11.4 b	78 de	7 f
2	25 PPM Zn from $ZnSO_4$	5.2 cd	148 a	29 c
	50 PPM Zn from $ZnSO_4$	3.0 de	144 a	48 b
	100 PPM Zn from $ZnSO_4$	2.1 e	185 a	88 a
	50 PPM Zn from ZnO	7.7 bc	151 a	20 d
	100 PPM Zn from ZnO	5.2 cd	160 a	31 c
	25 PPM from Zn chelate	3.9 de	172 a	44 b
	Gypsum at 0.3% by weight	7.9 b	69 b	9 e
	Biochar at 0.5% by weight	7.2 bc	54 b	8 ef
	Unamended soil	11.3 a	71 b	7 f

means within columns separated using Tukey's HSD test, $p<0.05$

[x] data were transformed to meet ANOVA assumptions

Table 4. Effects of Zn amendment on spinach dry tissue Cd concentration (PPM), and tissue Zn:Cd ratio, pot experiment 2.

	Soil 1		Soil 2		Soil 3	
Treatment	Cd	Zn:Cd[x]	Cd	Zn:Cd[x]	Cd	Zn:Cd[x]
80 PPM Zn whole pot	6.0 b	41.4 a	8.1 c	26.5 a	3.9 b	55.3 a
80 PPM Zn top 6" only	15.5 a	10.9 b	15.0 b	10.7 b	9.2 a	13.9 b
No Zn control	18.4 a	6.2 c	23.6 a	4.0 c	11.0 a	7.4 c

means within columns separated using Tukey's HSD test, $p<0.05$

[x] data were transformed to meet ANOVA assumptions

Table 5.Correlation of soil physiochemical characteristics with dry spinach tissue Cd concentration, pot experiment 3.

Soil characteristic	Range	Correlation coefficient (r)	Significance (p value)
pH	5.8 - 7.6	0.20	0.535
CEC (meq/100 g)	15 - 37	0.06	0.858
Organic matter (%)	1.3 - 3.5	-0.02	0.960
DTPA Cd (PPM)	0.3 - 1.9	0.80	0.002**
Total Cd (PPM)	0.5 - 5.4	0.86	0.001***
DTPA Zn (PPM)	1.0 - 11.0	-0.14	0.667
Total Zn (PPM)	35 - 104	0.38	0.228
DTPA Zn:Cd ratio	1.3 - 14.7	-0.73	0.008**
Total Zn:Cd ratio	15 - 105	-0.82	0.001**
Resin Cd (µg/capsule)	0.05 - 0.28	0.70	0.011*
Resin Zn (µg/capsule)	0.49 - 5.04	-0.40	0.195

*, **, *** correlation significant a $p< 0.05$, 0.01 and 0.0001, respectively

Table 6.Effects of Zn amendment on spinach dry tissue Cd concentration (PPM), and tissue Zn:Cd ratio, pot experiment 4.

	Soil 1		Soil 2		Soil 3	
Treatment	Cd	Zn:Cd^x	Cd	Zn:Cd^x	Cd	Zn:Cd^x
80 PPM Zn whole pot	7.4 b	29.3 a	9.6 c	26.6 a	3.3 c	55.2 a
80 PPM Zn top 4” only	10.3 b	16.2 b	22.5 b	7.0 b	8.4 b	13.4 b
No Zn control	22.1 a	4.4 c	27.2 a	3.9 c	9.9 a	7.7 c

means within columns separated using Tukey’s HSD test, $p<0.05$

^{x} data were transformed to meet ANOVA assumptions

Table 7.DTPA-extractable soil Zn (PPM) at the end of the first (pot experiment 2) and second (pot experiment 4) spinach crop.

	No soil Zn application		80 PPM Zn application	
Soil	end of 1st crop	end of 2nd crop	end of 1st crop	end of 2nd crop
1	2.8	3.0	50	41
2	3.0	2.8	55	46
3	2.4	2.7	49	44

Table 8.Effects of Zn and lime amendment of soil on spinach dry tissue Cd and Zn concentration (PPM), and Zn:Cd ratio, pot experiment 5.

Treatment	Description	PPM in leaf tissue: Cd	PPM in leaf tissue: Zn	Zn:Cd[x]
1	Control, unlimed	14.1 a	84 d	6.0 f
2	Control, limed	14.3 a	84 d	5.9 f
3	25 PPM Zn as $ZnSO_4$	5.9 de	122 abc	20.6 c
4	50 PPM Zn as $ZnSO_4$	3.8 e	142 a	38.0 a
5	25 PPM Zn as ZnO	9.3 b	108 cd	11.9 d
6	50 PPM Zn as ZnO	6.9 cd	126 abc	18.4 c
7	50 PPM Zn as $ZnSO_4$, limed	5.0 de	140 ab	27.8 b
8	50 PPM Zn pot average, all in top 3"	13.9 a	122 abc	8.9 e
9	50 PPM Zn pot average, all in top 6"	8.7 bc	110 bcd	12.7 d

means within columns separated using Tukey's HSD test, $p<0.05$

[x] data were transformed to meet ANOVA assumptions

Table 9.Effect of foliar zinc application on spinach leaf Cd and Zn concentration, pot experiment 6.

	Zinc	Pot trial soil 1		Pot trial soil 2		Field trial	
Treatments	applied lb/A	Cadmium mg/kg	Zinc mg/kg	Cadmium mg/kg	Zinc mg/kg	Cadmium mg/kg	Zinc mg/kg
Untreated	---	8.40	50.13	15.90	67.72	3.07	39.97
Zinc Sulfate 36%	0.5	8.09	212.80	15.88	231.50	3.32	99.50
Zinc Sulfate 36%	1.0	7.06	396.00	13.71	454.82	3.81	187.70
Zinc Chelate 9%	0.5	11.45	136.03	17.15	173.20	3.52	124.47
Zinc Chelate 9%	1.0	11.84	238.03	18.32	258.56	3.45	236.87
Pr>treatment		0.0003	<0.0001	<0.0001	<0.0001	0.01	<0.0001
LSD (0.05)		2.14	57.7	1.94	71.53	0.35	32.7

Table 10.Effect of irrigation water chloride (Cl) content on cadmium uptake in spinach, pot experiment 7.

Chloride concentration	Soil 1[1] Cd mg/kg	Soil 2[2] Cd mg/kg	Mean of both soils Cd mg/kg
0 ppm	5.32	10.12	7.72
50 ppm	5.31	10.96	8.13
100 ppm	6.40	11.43	8.92
200 ppm	7.78	15.18	11.48

[1]total cadmium level = 1.6 mg/kg; [2]total cadmium level = 2.3 mg/kg

Table 11.Spinach cadmium and zinc concentrations and soil characteristics at harvest, non-replicated large-scale field trial; first crop after soil treatments were applied. Standard deviation (st. dev.) represents subsample variability within soil treatments.

		Spinach tissue					Soil		
Zinc lbs/A	Amendment	Cadmium mg/kg DW	st. dev.	Cadmium mg/kg FW	Zinc mg/kg	st. dev.	DTPA Zinc mg/kg	pH	Organic Matter %
100	compost	6.9	1.1	0.58	99.0	3.9	17.8	7.3	1.32
100	compost + lime	6.9	0.4	0.58	95.9	7.4	18.4	7.4	1.47
100	lime	7.1	0.8	0.60	102.1	5.8	16.1	7.4	1.41
100	unamended	11.9	1.0	1.00	101.4	9.5	13.8	7.2	1.52
200	compost	5.5	0.7	0.46	122.7	13.3	36.7	7.3	1.34
200	compost + lime	4.1	0.2	0.35	104.6	8.7	33.3	7.5	1.56
200	lime	5.5	1.2	0.46	106.2	4.8	39.4	7.5	1.55
200	unamended	9.0	0.6	0.76	128.0	14.8	30.0	7.3	1.52

Table 12.Head lettuce cadmium and zinc concentrations and soil Zn level at harvest, non-replicated large-scale field trial; second crop after soil treatments were applied.

		Lettuce tissue			Soil
Zinc lbs/A	Amendment	Cadmium mg/kgDW	Cadmium mg/kgFW	Zinc mg/kgDW	Zinc mg/kg
100	compost	2.45	0.21	51.7	11.1
100	compost + lime	2.38	0.20	54.1	13.3
100	lime	2.87	0.24	46.7	11.0
100	unamended	3.53	0.30	49.0	10.2
200	compost	2.43	0.20	59.4	28.9
200	compost + lime	2.78	0.23	58.8	29.3
200	lime	2.74	0.23	56.9	30.8
200	unamended	3.61	0.30	59.5	22.0
0	Untreated	5.20	0.44	23.8	1.9

Table 13. Crop cadmium and zinc tissue concentrations and soil zinc level following an August, 2013, application of zinc fertilizers.

Treatment	Zn lb/A	Material	Spinach Oct 1, 2013 Tissue mg Cd/kg DW	Spinach Oct 1, 2013 DTPA soil Zn mg/kg	Broccoli April 3, 2014[1] Tissue mg Cd/kg DW	Broccoli April 3, 2014[1] Tissue mg Zn/kg DW	Broccoli April 3, 2014[1] DTPA soil Zn mg/kg
Untreated	---	---	14.2	4.6	0.50	46.9	3.0
Zn sulfate 36%	100	277 lbs	8.8	28.4	0.34	55.6	13.9
Zn sulfate 36%	200	554 lbs	7.6	60.8	0.31	66.6	38.4
Zn sulfate 36%	400	1108 lbs	7.3	103.6	0.32	81.5	55.8
Zn chelate 9%	100	100 gal	5.3	37.3	0.30	54.0	11.7
ActaGro 6.5% Zn	100	131 gal	9.0	28.4	0.35	54.8	---
6.0% Humic Acid	---	100 gal	14.2	3.5	0.52	44.6	---
Pr>F			<0.0001	0.0001	<0.0001	<0.0001	<0.0001
$LSD_{0.05}$			1.1	11.7	0.05	3.9	10.4
Observational treatment							
Zn sulfate 36%	800	2,216 lbs	7.8	159.2	0.36	91.1	---

1 – Soil DTPA Zn sample collected on Feb. 19, 2014

Table 13 (continued). Crop cadmium and zinc tissue concentrations and soil zinc level following an August, 2013, application of zinc fertilizers.

Treatment	Zn lb/A	Material	Baby redleaf lettuce June 26, 2014 DTPA soil Zn mg/kg	Baby redleaf lettuce June 26, 2014 Tissue mg Cd/kg DW	Baby redleaf lettuce June 26, 2014 Tissue mg Zn/kg DW	Head lettuce Oct 2, 2014 DTPA soil Zn mg/kg	Head lettuce Oct 2, 2014 Tissue mg Cd/kg DW	Head lettuce Oct 2, 2014 Tissue mg Zn/kg DW
Untreated	---	---	4.5	12.07	58.05	3.6	3.78	37.8
Zn sulfate 36%	100	277 lbs	6.8	11.06	78.97	6.5	2.96	42.55
Zn sulfate 36%	200	554 lbs	10.8	9.97	102.70	11.7	2.73	49.28
Zn sulfate 36%	400	1108 lbs	21.6	8.00	165.67	19.2	2.36	51.90
Zn chelate 9%	100	100 gal	7.9	10.53	88.15	7.3	2.87	41.45
ActaGro 6.5% Zn	100	131 gal	5.0	10.96	64.70	5.8	3.68	33.20
6.0% Humic Acid	---	100 gal	3.2	13.71	48.70	2.9	3.37	42.80
Pr>F			<0.0001	<0.0001	<0.0001	<0.0001	0.0225	0.0251
$LSD_{0.05}$			2.6	1.93	22.68	2.1	0.83	10.63
Observational treatment								
Zn sulfate 36%	800	2,216 lbs	27.2	8.44	165.20	22.0	2.08	63.8

Table 14.Effect of growth stage at harvest on spinach cadmium tissue concentrations.

Date	mg Cd/kg DW	mg Cd/kg FW	Stage of growth
Field 1			
July 30	0.99	0.08	1-2 true leaf
Aug 5	1.09	0.09	baby spinach
Aug 8	1.05	0.09	teenage spinach
Aug 13	0.70	0.06	freezer spinach
Field 2			
Aug 5	1.24	0.10	1-2 true leaf
Aug 8	1.36	0.11	baby spinach
Aug 13	1.28	0.11	teenage spinach
Aug 20	1.24	0.10	freezer spinach

Table 15.Effect of form of zinc on the uptake of cadmium and zinc by spinach, replicated field trial.

Material	Formulation	Zn lbs /A	Material applied lbs/A	Soil DTPA Zn (mg/kg)	Tissue mg Cd/kg DW	Tissue mg Cd/kg FW	Tissue mg Zn/kg DW
Untreated	---	---	---	1.43	3.65	0.31	44.47
zinc Sulfate 36%	powder	100	277.8	8.40	3.19	0.27	64.13
zinc Sulfate 36%	powder	200	555.6	18.37	2.96	0.25	68.47
zinc Sulfate 36% + $CaCO_3$	powder	200	555.6 4,000	15.97	2.86	0.24	71.97
zinc Sulfate 36%	granule	100	277.8	14.40	3.48	0.29	52.63
zinc Sulfate 36%	granule	200	555.6	23.70	3.42	0.29	59.43
zinc Oxide 72%	powder	100	138.8	3.30	3.50	0.30	57.13
zinc Oxide 72%	powder	200	277.8	7.57	3.09	0.26	60.90
Pr>treatment				0.0004	0.19	---	0.0002
LSD (0.05)				8.1	NS	---	8.5
Observational treat.							
$CaCO_3$	powder	---	4,000	1.20	3.82	0.32	46.80

Table 16.Effect of zinc formulation and rate on spinach cadmium and zinc concentrations, non-replicated strip trial.

Zinc lbs/A	Zinc Form	Tissue Cadmium mg/kg DW	Tissue Cadmium mg/kg FW	Tissue Zinc mg/kg
0	---	7.2	0.61	57.6
25	zinc Chelate 9%	2.0	0.17	93.1
100	zinc sulfate granules	6.4	0.53	96.8
100	zinc sulfate solution	4.0	0.34	170.0

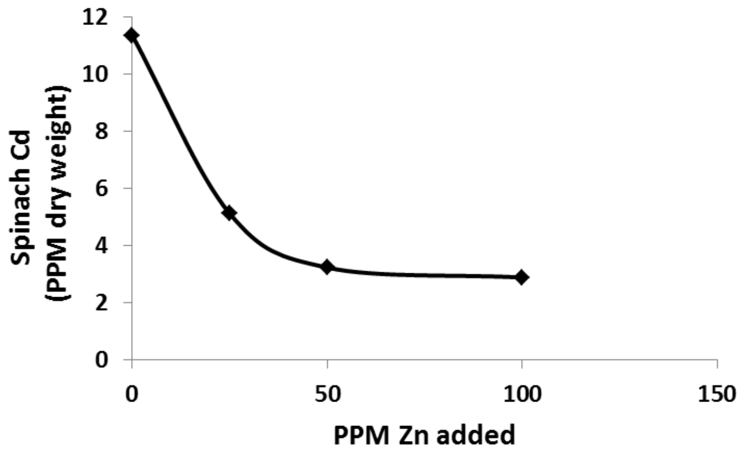

Fig. 1.Effect of $ZnSO_4$ amendment rate (PPM elemental Zn) to soil on spinach leaf Cd (PPM dry weight, across soils), pot experiment 1.