CALIFORNIA LETTUCE RESEARCH BOARD
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Title: Project title: Evaluation of Practices to Reduce Cadmium Uptake by Leafy Greens

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ABSTRACT:
Evaluations of two long-term field trials were continued in 2016 to observe the effect of zinc (Zn) applications on cadmium (Cd) uptake in various crops over time. The trials were established with cooperating growers and evaluations were made of crops in their normal rotations. Trial No. 1, established in 2014, showed that 100 lbs Zn/A as zinc sulfate reduced cadmium uptake by 30% in both head lettuce and peppers in 2016, three years after application. Prior to the trial, soil Zn levels were 1.9 ppm; after treatment, soil zinc levels increased to 11.4 ppm Zn in 2014 and were 10.4 ppm Zn in 2016. Trial No. 2 was established in 2015 showed that 100 lbs Zn/A as zinc sulfate reduced cadmium uptake by 38, 46 and 26% in arugula crop 1, spinach and arugula crop 2, respectively grown in 2016. These results indicate the level of effectiveness of Zn applications for mitigating the uptake of Cd by crops, and show that the effect can last at least three years, thereby allowing growers to amoritize the cost of application over a period of years. Two spinach variety trials were established to evaluate their differences in Cd uptake. There were significant differences in the uptake of Cd by varieties. Five varieties took up the lowest amounts of Cd in both trials. Pot studies were conducted to evaluate the impact of compost on Cd uptake. Compost had a weak but statistically significant effect on spinach Cd uptake, with tissue Cd concentration reduced by approximately 20% in the 10 ton/acre compost treatment compared to the control. Compost did not affect spinach Zn concentration. An evaluation of the impact of chloride (Cl) in irrigation water on the uptake of cadmium in spinach was conducted. Spinach tissue Cd concentration increased linearly with increasing irrigation water Cl concentration. The slope of the regression line showed that each meq/L increase in irrigation water Cl corresponded to a 0.25 PPM increase in dry tissue Cd concentration. This suggested that irrigation water quality is a potentially significant factor in spinach Cl uptake. Coastal irrigation wells typically range from approximately 1-8 meq/L Cl, occasionally higher. Fields irrigated with water at the top end of that range may have spinach Cd concentration more than 30% higher than if lower Cl water was used.

OBJECTIVES:

1. Evaluate the longevity of Zn, compost and lime and other amendments on Cd uptake by spinach and lettuce in on-going long-term trials in commercial production fields.
2. Evaluate the Cd uptake by spinach varieties and the extent to which varieties can be used to manage Cd issues in spinach production.
3. Conduct a detailed pot study that examines the effect of the combination of compost and zinc on cadmium uptake of spinach
4. Conduct a follow-up study on the impact of chloride on cadmium uptake by spinach

PROCEDURES:

Objective 1: Effectiveness and longevity of zinc applications. The goal of these trials was to make the zinc and soil amendment application strips large enough so we could sample the sites over a period of years without untreated soil being dragged into the sample area during normal tillage operations between crops. The growers grew their normal crop rotation during the evaluations and we sampled the soil and plant tissue at harvest of each crop to evaluate the impact on cadmium uptake and the longevity of the zinc applications. Three subsamples of tissue and soil were collected from each plot on each evaluation date. *Trial No. 1:* This trial was initiated with a cooperating grower near Greenfield on at site with 2.35 mg Cd/kg. The following amendments were applied to plots 280 feet long by 270 feet wide in May, 2014: 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. The materials were applied with a commercial applicator truck. Zinc at 100 and 200 lbs Zn/A (277 and 555 lbs zinc sulfate) was applied across the amendments in strips 53 feet wide by the length of the field (1100 feet). Zinc sulfate was solutionized to facilitate application to the soil and it was incorporated by discing. Four crops were grown in the 2014 and 2015, and in 2016, head lettuce and Anaheim pepper were grown, the 5th and 6th crops, respectively. *Trial No. 2:* This trial was initiated with a cooperating grower near King City on a site with 1.0 to 1.2 mg Cd/kg. The following amendments were applied to plots 165 long by 250 feet wide on May 5, 2015: 1) lime at the rate of 2 tons/A, 2) compost at 5 tons/A, 3) compost at 5 tons/A + lime at 2 tons/A, and 4) unamended. Across the amended areas a strip of zinc sulfate at the rate of 100 lbs Zn (280 lbs zinc sulfate) was applied on May 8, 2015 in an area 35 feet wide by the length of the field (990 feet). Two crops were grown in 2015 crop season, and in 2016, arugula, spinach and arugula were grown, the 3rd, 4th and 5th crops, respectively. Three observational trials were established adjacent to Trial No. 2: 1) an evaluation of two types of biochar applied at the rate of 6.5 tons/A on June 19, 2015 in plots 6 80-inch beds wide by 60 feet long; 2) zinc carbonate at 143 lbs Zn was applied June 15, 2015 in a strip 40 feet wide by the length of the field (990 feet); and 3) a strip of zinc oxide at 100 lbs Zn was applied on April 20, 2016 in a strip 40 feet side by the length of the field (990 feet).

Objective 2: Cadmium uptake by spinach varieties. Fifteen commonly used commercial spinach varieties were evaluated to observe differences in Cd uptake. *Trial No. 1.* This trial was established on a Cropley silty clay soil near Castroville. Soil cadmium levels were 0.2 ppm Cd (this level of soil Cd was lower than those commonly found in the Salinas Valley). The varieties were planted on August 15 on half of an 80-inch wide bed in a strip 10 feet long with a Sutton Junior planter. Each plot was replicated three times in a randomized complete block design. The plants were grown with standard commercial practices and were harvested on September 14. *Trial No. 2:* This trial was established on a Cropley silty clay soil near Gonzales. Soil cadmium levels were 0.9 ppm Cd. The varieties were planted on September 19 as described above. The plants were grown with standard commercial practices and were harvested on October 24. *Both Trials:* Tissue samples were collected at crop maturity and were washed and rinsed in deionized water, dried and sent to the UC Davis Analytical Lab for cadmium and zinc analysis.

Objectives 3 and 4: A loam soil from Monterey County was collected, air-dried and sieved through ¼" mesh. This soil, with a pH of 7.7 and 1.5% organic matter, contained 1.5 PPM total soil Cd. Two pot trials were conducted using this soil. Pots were 8" deep and contained approximately 3 kg of soil; they were fertilized with D-45 controlled release urea incorporated into the soil. Pots were seeded with 'Tambourine' spinach on June 2. On June 30 all vegetative biomass was harvested, oven-dried, ground and analyzed for Cd concentration. In each trial a randomized complete block experimental design was used, with 4 replicate pots per treatment.

Objective 3: Effect of compost and zinc application

To evaluate the relative effect of compost and zinc on suppression of spinach Cd uptake five treatments were compared:

- Control (no compost, no Zn)
- Compost @ 0.5% of soil weight
- Compost @ 1.0% of soil weight

- Compost @ 1.0% of soil weight + zinc sulfate @ 25 PPM elemental Zn
- Zinc sulfate @ 25 PPM elemental Zn

Compost treatments represented the equivalent of approximately 5 tons or 10 tons/acre if mixed into the top 6" of a field soil. The compost used was a commercially available material made from urban yard waste by a composter in the southern Salinas Valley. Both the compost and zinc sulfate were thoroughly blended into the dry soil before potting.

Objective 4: Effect of irrigation water chloride concentration

To evaluate the effect of irrigation water chloride (Cl) concentration on Cd uptake a trial was structured to evaluate 4 levels of irrigation water Cl (1, 4, 7 and 10 milliequivalents per liter, equal to approximately 35, 140, 280 and 350 PPM Cl). These solutions were made by adding Cl to a low Cl concentration water. Fifty percent of the Cl added was from NaCl and 50% from $CaCl_2$ to maintain a reasonable cation balance. All pots were germinated with the low Cl water, with the different levels of Cl beginning 7 days after seeding. Watering was controlled to ensure at least 10% of applied water leached from the pots, to minimize Cl buildup over the trial period; each pot utilized a 12" long fiberglass wick hanging from the bottom of the pot to improve drainage.

RESULTS:

Objective 1: Effectiveness and longevity of zinc applications. *Trial No. 1:* Although the trial is not replicated, there is a consistent trend that shows that zinc applications of 100 and 200 lbs Zn/A reduced cadmium uptake in the crops grown in 2016 (3rd year of the evaluation) (Table 1). 100 lbs Zn/A as zinc sulfate reduced cadmium uptake by 30% in both head lettuce and peppers in 2016. There is no indication that soil amendments provided further reduction of cadmium uptake in head lettuce and peppers. Original soil Zn levels were 1.9 ppm. Soil zinc levels increased to 11.4 ppm Zn in 2014 and were 10.4 ppm extactable Zn in 2016. *Trial No. 2:* Although the trial is not replicated, there is a consistent trend that shows that zinc applications of 100 lbs Zn/A reduced cadmium uptake in the crops grown in 2016 (the 2nd year of the evaluation) (Table 2). 100 lbs Zn/A as zinc sulfate reduced cadmium uptake by 38, 46 and 26% in arugula crop 1, spinach and arugula crop 2, respectively. There is no indication that soil amendments provided further reduction of cadmium uptake in these crops in 2016. Original soil Zn levels were 1.9 ppm. Soil zinc levels increased to 13.3 ppm in 2015 but declined to 8.5 ppm in the final crop of 2016. It is unclear if this decline was the result of the chemical nature of the soil or because the zinc treatment strip is narrower in this trial and that untreated soil may be reaching to the middle of the plots where soil samples are collected. In the observational trials, biochar mix 6 (the best biochar treatment) reduced cadmium uptake by 8.1% in the first arugula crop and 4.4% in the final arugula crop. Zinc carbonate at 143 lbs Zn/A reduced cadmium uptake by 44.5% in the first arugula crop and 10.6% in the final arugula crop. Zinc oxide reduced cadmium uptake by 31.7% in the first arugula crop and 6.2% in the final arugula crop. The drop in effectiveness of the Zn application between the first and second crops may reflect the lower solubility of these materials; the treatment strips are wider than the zinc sulfate strip referred to above and it is unlikely that dragging of untreated soil is a factor in these evaluations. These results indicate that zinc sulfate provides greater reductions in Cd uptake by crops in the short and long-term.

Objective 2: Cadmium uptake by spinach varieties. Two trials were conducted evaluating the cadmium uptake of spinach varieties. Trial no. 1 was conducted on a site with 0.2 ppm Cd which is quite low compared to many other sites in the Salinas Valley (we had placed the trial at this site under the assumption it was a normal Salinas Valley site and we got the soil results back we realized that it was lower than we would have wanted). Trial No. 2 had 0.9 ppm Cd which is fairly typical of soils located on the valley floor of the Salinas Valley formed by river sediments. There were significant differences in Cd uptake by the varieties. The five lowest uptake varieties occurred in both trials (Table 3). Of the high uptake varieties on the 0.9 ppm Cd site, some were low on the 0.2 ppm Cd site indicating that they are less capable of dealing with high Cd soils.

Objective 3: Effect of compost and zinc application. Neither compost nor Zn significantly affected spinach growth (Table 1). Compost had a weak but statistically significant effect on spinach Cd uptake, with tissue Cd concentration reduced by approximately 20% in the 10 ton/acre compost treatment compared to the control. Compost did not affect spinach Zn concentration. Amending the soil with zinc sulfate, whether alone or in combination with compost, had a much stronger effect on Cd uptake, with tissue Cd concentration declining by approximately 55% compared to the control. Zn application also increased the tissue Zn concentration, thereby dramatically increasing the Zn:Cd ratio. The Zn:Cd ratio has human health significance because elevated plant Zn concentration reduces Cd absorption in the human digestive system.

Table 1. Effect of soil incorporation of yard waste compost and zinc sulfate on spinach tissue cadmium and zinc concentrations.

	Dry wt	PPM dry wt		
	(g/plant)	Cd	Zn	Zn:Cd ratio
Control (no compost, no Zn)	4.2 a	5.7 a	52 b	9 b
5 tons/acre compost	4.6 a	5.2 ab	59 b	11 b
10 tons/acre compost	4.4 a	4.6 b	56 b	12 b
10 tons/acre compost + 25 PPM Zn	4.6 a	2.6 c	100 a	38 a
25 PPM Zn	4.2 a	2.6 c	92 a	35 a

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

Objective 4: Effect of irrigation water chloride concentration. Spinach tissue Cd concentration increased linearly with increasing irrigation water Cl concentration (Fig. 1). The slope of the regression line showed that each meq/L increase in irrigation water Cl corresponded to a 0.25 PPM increase in dry tissue Cd concentration. This suggested that irrigation water quality is a potentially significant factor in spinach Cl uptake. Coastal irrigation wells typically range from approximately 1-8 meq/L Cl, occasionally higher. Fields irrigated with water at the top end of that range may have spinach Cd concentration more than 30% higher than if lower Cl water was used.

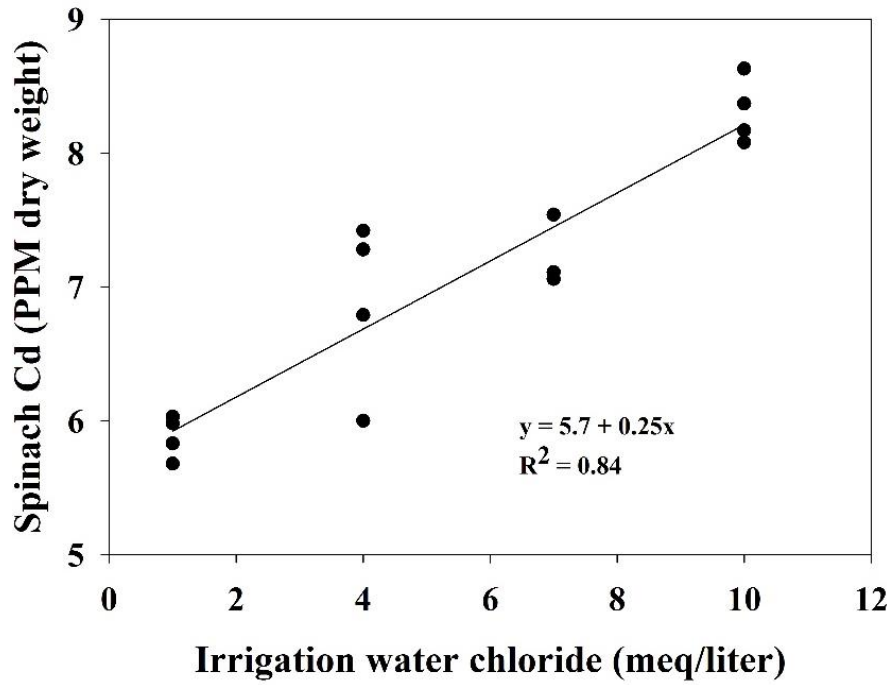

Fig. 1. Effect of irrigation water Cl concentration on spinach tissue cadmium concentration.

Table 1. Trial No. 1. Cadmium and zinc evaluations of zinc and amendment trial near Greenfield. Soil Cd 2.35ppm.

Zinc lbs/A	Amendment	April 12, 2016						September 23, 2016			
		Head lettuce tissue			Head lettuce soil			Anaheim pepper fruit tissue			Soil
		Cadmium mg/kg DW	Cadmium mg/kg FW[3]	Zinc mg/kg DW	Zinc mg/kg	pH	Organic Matter %	Cadmium mg/kg DW	Cadmium mg/kg FW[4]	Zinc mg/kg DW	Zinc mg/kg
0	compost	5.75	0.24	32.7	3.9	7.59	1.6	1.86	0.228	21.17	3.47
0	compost + lime	7.30	0.30	26.0	3.5	7.64	1.7	2.16	0.265	21.10	3.20
0	lime	8.07	0.33	28.8	3.3	7.44	1.7	2.07	0.254	19.80	3.07
0	unamended	8.96	0.37	30.4	2.7	7.53	1.6	2.01	0.246	22.30	2.93
100	compost	4.79	0.20	45.2	13.1	7.52	1.6	1.44	0.176	22.77	10.47
100	compost + lime	4.77	0.20	44.6	12.5	7.53	1.7	1.57	0.192	21.20	10.77
100	lime	5.31	0.22	48.5	11.1	7.73	1.7	1.40	0.171	20.60	11.10
100	unamended	5.25	0.22	50.2	9.9	7.39	1.5	1.38	0.169	20.73	9.13
200	compost	3.81	0.16	52.3	25.3	7.22	1.6	1.58	0.194	24.40	22.40
200	compost + lime	3.23	0.13	52.6	23.2	7.54	1.9	1.41	0.172	24.70	18.17
200	lime	3.70	0.15	63.3	25.1	7.58	1.7	1.25	0.153	22.97	17.93
200	unamended	4.09	0.17	64.9	23.8	7.50	1.7	1.35	0.165	23.30	17.87

3 – the conversion factor from dry weight to fresh weight was 24.33; 4 – the conversion factor from dry weight to fresh weight was 8.16

Table 2. Trial No. 2. Cadmium and zinc evaluations of zinc and amendment trial near King City. Soil Cd ranged from 1.0 to 1.2 ppm.

Zinc lbs/A	Amendment	March 17, 2016				June 9, 2016			
		Arrugula			Soil	Spinach			Soil
		mg Cd/kg DW	mg Cd/kg FW	Zn mg/kg DW	Zinc mg/kg	mg Cd/kg DW	mg Cd/kg FW	Zn mg/kg DW	Zinc mg/kg
0	Unamended	1.48	0.12	36.2	1.7	3.45	0.29	51.3	1.7
0	Compost	1.59	0.13	38.2	1.9	3.54	0.30	49.4	1.8
0	Lime	1.56	0.13	37.8	1.9	3.87	0.33	48.7	1.9
0	Compost + Lime	1.47	0.12	36.8	1.8	3.51	0.30	50.0	1.8
100	Unamended	0.92	0.07	45.7	9.3	1.78	0.15	58.3	10.1
100	Compost	1.02	0.09	47.7	10.1	190	0.16	59.8	8.3
100	Lime	0.98	0.08	46.2	9.4	2.05	0.17	64.3	9.7
100	Compost + Lime	0.89	0.07	45.6	12.1	2.08	0.17	57.0	7.4
0	No biochar	1.60	0.13	41.0	1.7	2.43	0.20	44.0	1.5
0	Biochar mix 5	1.50	0.13	40.7	1.6	3.33	0.28	55.1	1.5
0	Biochar mix 6	1.47	0.12	41.5	1.4	2.92	0.25	46.7	1.4
	Other zinc evaluations								
0	Untreated	---	---	---	2.0	3.28	0.28	38.2	3.4
143	Zinc Carbonate	---	---	---	12.6	1.82	0.15	69.5	9.7
100	Zinc Oxide	---	---	---	---	2.24	0.19	78.4	9.8

Table 2. Trial No. 2. Continued.

Zinc lbs/A	Amendment	Sept 11, 2016			
		Arugula			Soil
		mg Cd/kg DW	mg Cd/kg FW[1]	Zn mg/kg DW	Zinc mg/kg
0	Unamended	2.14	0.20	27.5	1.7
0	Compost	2.34	0.22	27.2	1.7
0	Lime	2.28	0.21	27.5	1.8
0	Compost + Lime	2.45	0.23	27.8	1.8
100	Unamended	1.61	0.15	81.1	8.8
100	Compost	1.72	0.16	78.4	9.2
100	Lime	1.71	0.16	75.6	8.4
100	Compost + Lime	1.73	0.16	73.2	7.9
0	No biochar	2.23	0.21	28.4	1.7
0	Biochar mix 5	2.16	0.20	27.9	1.5
0	Biochar mix 6	2.13	0.20	27.1	1.4
	Zinc carbonate evaluation				
0	Untreated	2.07	0.19	35.1	2.4
143	Zinc Carbonate	1.85	0.17	102.5	10.6
100	Zinc Oxide	1.94	0.18	71.1	5.0

1 – the conversion factor from dry weight to fresh weight was 10.87 (estimated from FDA Moisture Content Guidelines for mustard greens)

Table 3. Two spinach variety trials sorted from lowest to highest concentration of Cd in tissue

Trial 1 (soil = 0.2 ppm Cd)				Trial 2 (soil = 0.9 ppm Cd)				Average		
Variety	mg Cd/kg DW	mg Cd/kg FW		Variety	mg Cd/kg DW	mg Cd/kg FW		Variety	mg Cd/kg DW	mg Cd/kg FW
Sparrow	2.05	0.17		Sparrow	2.76	0.23		Sparrow	2.40	0.20
Callisto	2.16	0.18		Violin	2.98	0.25		Callisto	2.77	0.23
Amazon	2.54	0.21		Amazon	3.28	0.28		Violin	2.81	0.24
Piano	2.55	0.21		Callisto	3.39	0.28		Amazon	2.91	0.24
Violin	2.64	0.22		Piano	3.49	0.29		Piano	3.02	0.25
Sioux	2.74	0.23		Seaside	3.68	0.31		Meerkat	3.68	0.31
Tambourine	3.01	0.25		Meerkat	3.78	0.32		Seaside	3.69	0.31
Woodpecker	3.03	0.26		Silverwhale	4.56	0.38		Tambourine	3.80	0.32
Tasman	3.14	0.26		Tambourine	4.58	0.39		Woodpecker	3.81	0.32
Riverside	3.17	0.27		Woodpecker	4.59	0.39		Silverwhale	3.92	0.33
Silverwhale	3.28	0.28		Banjo	4.87	0.41		Sioux	4.00	0.34
Banjo	3.31	0.28		Riverside	5.05	0.43		Banjo	4.09	0.34
Meerkat	3.59	0.30		Carmel	5.13	0.43		Riverside	4.11	0.35
Carmel	3.65	0.31		Tasman	5.14	0.43		Tasman	4.14	0.35
Seaside	3.70	0.31		Sioux	5.26	0.44		Carmel	4.39	0.37
P	<0.0001	<0.0001			<0.0001	<0.0001			---	---
$LSD_{0.05}$	0.43	0.04			0.49	0.04			---	---