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A STUDY ON THE ACCUMULATION OF PERCHLORATE IN YOUNG HEAD LETTUCE

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PREFACE

The Colorado River is contaminated with perchlorate at low levels (5-9 parts-per-billion, ppb). Much of the lettuce consumed in the winter months in the U.S. is irrigated by Colorado River water. Results from 5,650 drinking water sources in California show perchlorate detections in only 319 sources above the reporting level of 4 μg/L (parts-per-billion, ppb). However, perchlorate levels of up to 260 ppb were detected in wells near weapons manufacturing facilities in Sacramento and Los Angeles counties. Perchlorate has also been detected at levels of 17 ppb in Lake Mead as a result of releases from two ammonium perchlorate manufacturing facilities in Nevada. The primary sources of perchlorate contamination appear to be from industrial and military operations that use perchlorate as an oxidizing agent. Perchlorate contamination in water is of concern because of uncertainties about toxicity and health effects from low levels in drinking water sources, the impact on ecosystems, and possible indirect exposure pathways to humans from agricultural and other activities. Anion exchange resins, microbial-mediated reduction, and phytoremediation are under investigation as ways to remove perchlorate from contaminated waters.

Phytoremediation is the use of plants to remove both ppb and parts-per-million (ppm) levels of organic and inorganic pollutants from contaminated soil and water. Since 1999 research into the ability of terrestrial and aquatic plants to degrade or accumulate perchlorate has been reported by several groups including EPA/NERL-Athens. The Athens researchers observed accumulation of perchlorate in aquatic species such as blue-hyssop and parrot-feather that suggested that leafy vegetables such as cabbage and lettuce might accumulate perchlorate. For these reasons, potential accumulation of perchlorate in lettuce leaves was identified as one of six high-priority research needs at the U.S. Air Force's (USAF) Little Rock Eco Summit in April 1999. This study was part of the work plan of an interagency agreement between the USAF and EPA to investigate the fate of, and potential exposures to, perchlorate. This greenhouse study was designed as a narrow screening-level test to determine the degree of perchlorate uptake (from fortified nutrient solution) and subsequent accumulation in lettuce leaves.

The report was submitted for review, consistent with a level 2 EPA product. Specifically, this report underwent five external technical reviews, one internal editorial review, and one internal QA review. Overall, most reviewer comments were calls for more information and details. Incorporating the requested information produced this modified report that more clearly communicates the important finding that lettuce accumulates perchlorate from fortified nutrient solution. However, follow-up studies will be required before this potential perchlorate exposure route can be fully characterized. This successful demonstration of the uptake and accumulation of perchlorate by lettuce in the greenhouse study is information that other researchers can use in further research on uptake of perchlorate by lettuce grown under field conditions. EPA/NERL-Athens concluded research on perchlorate in June 2002 and does not plan any further research on perchlorate.

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1.0 INTRODUCTION

Perchlorate (ClO_4^-) releases have been confirmed in 20 states throughout the United States. The majority of the releases are in California, Nevada, Arizona, and Texas[1,2]. In California, detections have been primarily in groundwater sources in the counties of Los Angeles, San Bernardino, and Riverside, as well as sources containing water from the Colorado River[3]. Results from 5,650 drinking water sources in California show perchlorate detections in only 319 sources above the reporting level of 4 μg/L (parts-per-billion, ppb). Perchlorate levels of up to 260 ppb were detected in wells near weapons manufacturing facilities in Sacramento and Los Angeles counties[4]. Perchlorate has also been detected at low levels (5-9 ppb) in the Colorado River[5] and up to 17 ppb in Lake Mead as a result of releases from two ammonium perchlorate manufacturing facilities in Nevada[2].

The primary sources of perchlorate contamination appear to be from industrial and military operations that use perchlorate as an oxidizing agent[6]. Perchlorate is water soluble, exceedingly mobile in aqueous systems, and can persist for many decades under typical ground and surface water conditions[6]. Perchlorate contamination in water is of concern because of uncertainties about toxicity and health effects from low levels in drinking water sources, the impact on ecosystems, and possible indirect exposure pathways to humans from agricultural and other activities[1]. Anion exchange resins, microbial-mediated reduction, and phytoremediation are under investigation as ways to remove perchlorate from contaminated waters[1,5,7].

Phytoremediation is the use of plants to remove both ppb and parts-per-million (ppm) levels of organic and inorganic pollutants from contaminated soil and water. The concentrations ppb and ppm differ by a factor of 1,000 i.e., 1 ppm = 1,000 ppb. Since 1999 research into the ability of terrestrial and aquatic plants to degrade or accumulate perchlorate has been reported by several groups[7-11]. A large part of the national supply of winter fruits and vegetables, including lettuce, are grown in southern California and Arizona and are irrigated with Colorado River water[12]. Currently, there are very limited data about the possible uptake of perchlorate into agricultural products caused by irrigation with low ppb levels of perchlorate-contaminated water. Accumulation of perchlorate in aquatic species such as blue-hyssop and parrot-feather[7] suggested that leafy vegetables such as cabbage and lettuce might accumulate perchlorate.

The overall objective of this study therefore was to demonstrate in a greenhouse study the potential for incorporation of perchlorate from aqueous solutions of 10, 50, 100, 500, 1,000, 5,000, and 10,000 ppb into an agricultural food crop (lettuce; *Lactuca sativa*), which is typically grown under irrigated conditions. A sand matrix amended with water containing known amounts of perchlorate was used as the growth medium to accentuate uptake. The successful demonstration of the uptake and accumulation of perchlorate by lettuce in the greenhouse study was seen as information that other researchers could use in further research on uptake of perchlorate by lettuce grown under field conditions.

2.0 EXPERIMENTAL METHODS

The growing conditions described in the standard method for conducting seedling growth tests (ASTM E1598-94)[13] were used as a guideline for plant growth throughout the study. Lettuce plants (*Lactuca sativa*) were grown from seed (Burpee's Iceberg Crisphead, Packaged for 2000 Lot 1, W. Atlee Burpee & Co., Warminter, PA, 90-120 days to maturity) in conical plastic containers, 14 cm deep and 3.8 cm in diameter at the top. The conical containers held approximately 135 g washed sand and were used for the first 51 days of the experiment. On day-51 the plants were repotted, without disrupting the root ball, by transferring the plant and the sand from the smaller containers into10 cm x 10 cm x 8 cm plastic containers with approximately 550 g of additional washed sand for expanded root growth. In both containers the sand was within 2 cm of the top of the container. The bottoms of both types of containers were lined with glass wool to prevent loss of sand from the containers.

The plants were grown in a greenhouse in Athens, GA from the last week in February until the first week in June 2000. Germination (appearance of the first leaf above the sand surface) occurred at day-7. The day-95 samples were collected 95 days after germination for a total study duration of 102 days from seeding. The greenhouse was not temperature controlled, but was equipped with an electric fan that was installed opposite a screen door to provide cross-ventilation airflow. The fan was controlled by a thermostat set to activate the fan at 29.5 ^{0}C (85 ^{0}F). Additionally, the greenhouse was equipped with fluorescent grow lights that were operated 14 hrs per day throughout the study to enhance light intensity and increase the photoperiod.

Children's play box sand was purchased locally and washed with tap water until the wash water was clear. A sample of the last wash water was analyzed for perchlorate by ion chromatography.

Plant nutrient solution was prepared from Peter's Professional plant food (20-20-20, 1.9% nitrate nitrogen) purchased locally in Athens, GA. The nutrient solution was prepared per directions stated on the bag by adding 3.5 g grab sample of the solid to 1 L of 18 MΩ water and mixing thoroughly. The nutrient solution was prepared as needed. Before the start of the study, the first preparation of nutrient solution was analyzed for perchlorate before application to the plants. The plants were fertilized once per week throughout the study with 5 mL of the nutrient solution by slowly releasing the nutrient solution from a pipette into the sand at the base of the plant.

Seeds were germinated with application of water as needed to keep the sand moist and once per week 5 mL of the nutrient solution was added to all the plants; beginning when the seedlings were 14 days old, plants were also watered with 10, 50, 100, 500, 1,000, 5,000 and 10,000 ppb solutions of perchlorate. The treatment solutions were made one time by weighing the calculated amount of solid sodium perchlorate (Fisher Scientific) into a 500-mL volumetric flask and bringing to volume with18 MΩ water. Nutrient and perchlorate treatment solutions were added at a rate to prevent dripping from the bottom of the containers. Perchlorate treatment

solution was applied to the containers three times per week at a maximum of 10 mL/day. To maintain plant health, supplemental watering was done with tap water on days during the latter stages of growth when no perchlorate solution was added. The final perchlorate treatment was day-93.

Based on the analysis of three lettuce plants at seven perchlorate treatment levels plus a control for 12 sampling dates, the total number of plants sampled during the study was 288. In the greenhouse, the plants for each treatment level and control were grouped together in a shallow container and weekly the groups were rotated to different locations in the greenhouse. During the study, the plants were spaced such that leaves of adjacent plants did not touch to allow air circulation. On designated days during the study, the perchlorate content of the leaves and roots at each level of treatment was measured in three separate lettuce plants. The first sampling event occurred on day-21 from seeding (14 days after germination) with the final day-95 sampling occurring 102 days from seeding. Three control plants were also analyzed at each sampling event. Each lettuce plant was separated into above (leaf)- and below (root)-ground biomass before analysis. The day-86 and day-95 samples had small heads that were separated from the outer leaves and the inner (head) and outer leaves were analyzed separately.

A published method[14] was modified and used for the extraction of plant tissue and for instrumental analysis of an aliquot of water from the last sand wash, the nutrient solutions, and the aqueous extracts of the lettuce leaves and roots. One modification entailed oven drying and pulverizing the dried plant tissue rather than freeze-drying and grinding. Additionally, the analytical column was 4 mm internal diameter (ID) rather than 2 mm and the injected volume was 100 µL rather than 1,000 µL. The fresh plant tissue was weighed, washed with 18 MΩ water, and dried at 104^{o}C for 24 hours in uncapped glass vials. The tissue dry weight and percent moisture were recorded for each sample. Perchlorate was extracted from the dry and pulverized plant material with 18 MΩ water at an approximate mass to volume ratio of 0.6 g to 30 mL depending on the whole plant dry mass. Water (18 MΩ) was added and the vials were capped and placed in a boiling water bath for 30 minutes. The vials were cooled and stored at 4°C for 24 hrs. The aqueous extract was filtered through 1 layer of Kimwipes and the filtrate was centrifuged at 20,000 x g for 30 min to remove any residual plant tissue. Organic acids and interfering ions were removed from the supernatant by adding 0.5 g of DD-6 alumina per mL of extract. The extract was allowed to remain on the DD-6 for 24 hr at 4^{o}C. An aliquot was removed, filtered through a 0.4 micron Acrodisc® filter, and analyzed for perchlorate using an isocratic ion chromatographic (IC) procedure. The sand and glass wool in the plant containers were discarded and not analyzed for perchlorate.

A Dionex Ion Chromatograph (IC) equipped with a GP40 gradient pump, AD20 absorbance detector, CD20 conductivity detector, AS3500 autosampler, and LC20 chromatography enclosure was used for analysis of perchlorate in the tissue and water samples. Ion analysis was performed with an Ionpac AS 16-HC (4-mm X 250 mm) analytical column. A guard column preceded the analytical column to prevent sample contaminants from eluting onto the analytical column. The column flow rate of eluent (sodium hydroxide 50 mM) was 1.0 mL min^{-1}. The injection loop volume was 100 µL, and the run time for perchlorate analysis was 20 min. An anion self-regenerating suppressor (ASRS) was used for suppressed-conductivity detection. Distilled and deionized water was used for regeneration of the ASRS. Standard

curves were calculated from injection of 500 ppb to 25,000 ppb calibration standards. Based on injection of 100 μL injections, the instrument detection limit was 300 ppb. The estimated method detection limit was 500 ppb (25 ppb on a dry weight basis) based on the quantification of the extracts of perchlorate-fortified lettuce.

The first nutrient solution prepared from each bag of Peter's plant food and a sample of the water collected from the final washing of the sand were analyzed on this IC after filtration through a 0. 45 ③m filter. After detection of perchlorate in the control samples midway through the study, the most recently prepared nutrient solution and the sand washing sample were analyzed on a second IC with a lower limit of detection (see Results and Discussion).

At each sampling event, three plants of equal size were removed from the green house and analyzed for perchlorate. The remaining plants were rearranged to maintain equal spacing and airflow. The plant dry mass and perchlorate concentration means and standard deviations from the analysis of the three plants at each sampling event were used to plot plant accumulation of dry mass over time as well as total perchlorate accumulation in the roots and leaves of the entire plant at each sampling event.

3.0 RESULTS AND DISCUSSION

The photoperiod, temperature, water, and nutrient conditions to which the plants were exposed in the greenhouse were sufficient to maintain steady biomass accumulation (Figure 1). The greenhouse was not heated. The lowest recorded temperature during the study was 10ºC and the highest 35ºC. When the ambient temperature in the greenhouse reached 29.5 ^{0}C (85 ^{0}F) a fan was automatically activated to circulate air and to moderate the temperature. Based on information supplied with the seed, the time to maturity for the Crisphead lettuce is 90-120 days. The day-95 plants had well defined heads and the typical green color of lettuce. Dry mass accumulation of the lettuce with respect to days from seeding is shown in Figure 1 starting from day-21. It is evident from Figure 1 that the biomass accumulation of all the plants was similar. Exposure to perchlorate, even at 10,000 ppb, did not affect plant growth and there was no visible or textural difference in the10,000 ppb plants and the control plants.

For consistency in lettuce sample size, at each sampling event three plants of similar size were chosen from each treatment level and control plants. The drop in biomass for the day-72 and day-86 samples could be attributed to the fact that at this time in the study the overall number of plants at each treatment level was reduced to a level such that only smaller plants of similar size remained. The last perchlorate treatment was on day-93. The almost doubling in plant mass between day-86 and day-95 samples was attributed to the supplemental water that was added to the plants daily for the latter days of the study. This additional water, plus continued root expansion, possibly allowed the roots to grow and transpire nutrients that had been deposited out of the root zone during the previous wetting/drying events. Since perchlorate would migrate with the nutrients, deposition of perchlorate outside the root zone is one explanation for the less than 100 % recovery of perchlorate reported in Table 1.

Even though the water from the last sand washing and each weekly nutrient solution were tested for perchlorate, perchlorate was observed in the control plants in the day-35 samples. The

nutrient solution that had been prepared for the post day-35 plants and the sand-washing sample were reanalyzed on a second IC with a lower detection limit (1-4 ppb). The tap water sample from the sand washing did not contain perchlorate; this fact also indicated the tap water used to water the plants in the latter stages of the study did not contain perchlorate. However, the Peter's nutrient solution was found to contain 100 ppb perchlorate. The addition of perchlorate from the nutrient solution was considered to be constant throughout the study and included in the calculation of the total mass of perchlorate added to the plant at each perchlorate treatment level (Table 1). As can be seen in Table 1, the perchlorate added via Peter's nutrient solution contributed a small fraction of the total amount of perchlorate added to treatments 1,000, 5,000, and 10,000 ppb perchlorate. However, in the lower concentrations (100 to 500 ppb), the amount of perchlorate added in the Peter's solution ranged from approximately 30% in the 100 ppb treatment to around 6% of the 500 ppb treatment. The 10 and 50 ppb treatment level data were not reported due to the high percentage of perchlorate added by the nutrient solution.

Post day-35 preparations of nutrient solution were not analyzed for perchlorate, but the solutions were prepared from the same bag as was used to prepare the nutrient solution that contained 100 ppb perchlorate. Since each preparation of nutrient solution was essentially a grab sample from a heterogeneous mixture of solid ingredients the perchlorate concentrations of the nutrient solutions may not have been constant at 100 ppb. Thus more or less than the calculated amount of perchlorate may have been added than was accounted for based on the single measurement. This is evident in Table 1 where the mass of perchlorate recovered from the control plants on days 72, 86, and 95 was much greater than the corresponding calculated amount.

In the 100 ppb and higher treatment samples, measurable perchlorate was observed in the above-ground biomass (leaves) on day-21 (Figure 2 and Table 1). The first lettuce samples were collected and analyzed for perchlorate seven days after the initial perchlorate treatments. In Figure 2 the perchlorate concentration in the leaf dry mass appeared to be concentration dependent and increased steadily with time over the first 6 to 7 weeks of growth. Also, in Figure 2, the decline in the perchlorate concentration after day 51 may be related to the repotting, which occurred at this time. As seen in Figure 1, the plant biomass increased at a steeper rate for the day-58 and day-65 samples; the increased rate of biomass is reflected in the decline in the Figure 2 day-58 and day-65 perchlorate concentration data.

A second sudden increase in plant biomass is evident in Figure 1 between the day-86 and day-95 samplings. Because of high daily temperatures experienced in the latter stages of the study, the volume of additional non-contaminated water added each week increased but was added at a rate that did not cause dripping from the bottom of the container. The amount of perchlorate-amended water was kept at 10 mL per application, 3 days per week to ensure no loss of perchlorate due to leaching. The additional water enhanced plant growth and yielded an increased level of dry biomass in the day-95 samples. Possibly the growth spurt was caused by the extra water solubilizing accumulated nutrient solution and making it available to the roots. The increase in the day-95 dry biomass diluted the accumulated perchlorate, compared to the day-86 concentrations, as shown in the Figure 2 dry biomass concentration of perchlorate in the day-95 samples. In Figure 2, the concentrations ranged from a maximum of 3,600,000 ppb dry plant material (480,000 ppb wet plant material) with the addition of 10,000 ppb perchlorate

solution to a low accumulation of 90,000 ppb dry plant material (5,000 ppb wet plant material) in the 100 ppb treatment.

In Figure 3 total perchlorate extracted from the plant tissue was plotted against total perchlorate added in solution (data also in Table 1). From this graph, it is obvious that perchlorate uptake was continuous throughout the plant growth cycle. The calculated percent recovery of perchlorate from each treatment based on best-fit linear regression lines through the entire data set (days 14-95, Figure 3) is reported in Table 2. The coefficient of determination was included as an indicator of best-fit line. At treatment levels of 500 ppb to 10,000 ppb, the amount of uptake into the above-ground biomass in Table 2 accounts for 73 to 82% of the applied perchlorate. This would indicate that perchlorate was carried with the transpiration stream and was potentially 100% translocated at these concentrations.

Percent recovery from the final takedown (day 95) is listed in Table 3. Perchlorate recovered from treatments 500-10,000 ppb was similar to the calculated values reported in Table 2 and ranged from 66 to 90% of the applied perchlorate. The perchlorate content determined in the roots of the 10,000 ppb treatment on day 95 was 1.3% (30 μg) of the applied amount. The 30 μg of perchlorate recovered in the 10,000 ppb day-95 samples was the largest amount recovered in roots in any samples during the study. At all the treatment levels, the perchlorate not accounted for in the above ground biomass and the roots was assumed to be in the sand/glass wool that remained in the container when the plant was removed. The method[14] used for the extraction of perchlorate has been shown to quantitatively recover perchlorate from lettuce tissue. The boiling water bath extraction does not degrade perchlorate but disrupts cell walls and liberates bound perchlorate.

By the final takedown (day 95 from planting), the plant was large enough to separate the older, outer leaves from the inner leaves, which had formed a small lettuce head. These small lettuce heads (inner leaves) were analyzed separately from the older outer leaves. The inner head and outer leaf perchlorate concentrations and concentration factors (CFs) are shown in Table 4. The CFs were calculated by dividing the wet plant concentration by the treatment concentration. For the four treatment levels the wet plant concentration (ppb) of perchlorate in the outer leaves was from 2 to 6 times higher than the inner leaves. These data indicate that more perchlorate is accumulated in the older, outer leaves of the lettuce plant with lower concentrations in the newly formed inner leaves (head). Because the majority of water transpired by lettuce during head formation and growth is through the exposed outer leaf material, these results strongly suggest that perchlorate moves with the transpiration stream and the mature inner head will contain substantially less perchlorate. Specifically, these data show that the inner lettuce head that was not exposed to light, and also transpired substantially less water, resulted in concentration factors that were 68% to 89 % less than the outer exposed leaf material, depending on treatment level.

4.0 SUMMARY AND CONCLUSIONS

The irrigation study indicates that perchlorate was transported from the root zone and incorporated into lettuce leaf tissue. The recovery values for the 500 ppb and higher treatments were approximately 79% (average of Table 3 recovery values); this suggests the majority of the

perchlorate was incorporated into plant tissue and remained there until the end of the study. The unaccounted for 21% possibly remained in the sand, which was not analyzed after removal of the intact plant; the lettuce tissue extracts were not analyzed for products of degradation. Another possibility is that because of the weekly additions of nutrient solution nutrient anions accumulated and competed with perchlorate for uptake. The study also suggested that the uptake into lettuce was related to mass transport into the plant as driven by transpiration. Passive mass transport uptake of perchlorate was reported in a recent study that showed perchlorate was quantitatively transported via the transpiration stream to tobacco leaf from hydroponics nutrient solution[15].

The data from this bench-scale greenhouse study indicate that perchlorate is accumulated in the older, outer leaves of the lettuce plant, with significantly lower concentrations in the newly formed inner leaves (head). The CFs derived by calculating the wet plant concentration by the treatment concentration are17-28 for the outer leaves, and 3-9 in the emerging head. The greenhouse conditions in this study did not mimic field conditions and the perchlorate-fortified treatment solution was added in a way designed to maximize uptake. Thus, the accumulation of perchlorate was possibly enhanced over what would be accumulated in lettuce grown in the field and irrigated with perchlorate-contaminated water.

Recent reports have shown that perchlorate does not appreciably sorb to soils and that its mobility and fate in surface and groundwater are largely influenced by the flow of the water and the presence or absence of organisms that degrade perchlorate[1,5,16]. Another study showed that perchlorate that was present as a natural constituent in the applied fertilizer accumulated in leaf of tobacco grown under field conditions[17]. These recent reports support the potential for lettuce uptake of perchlorate grown under field conditions, but follow-up studies are required before one can fully characterize this exposure route in the above-ground vegetation.

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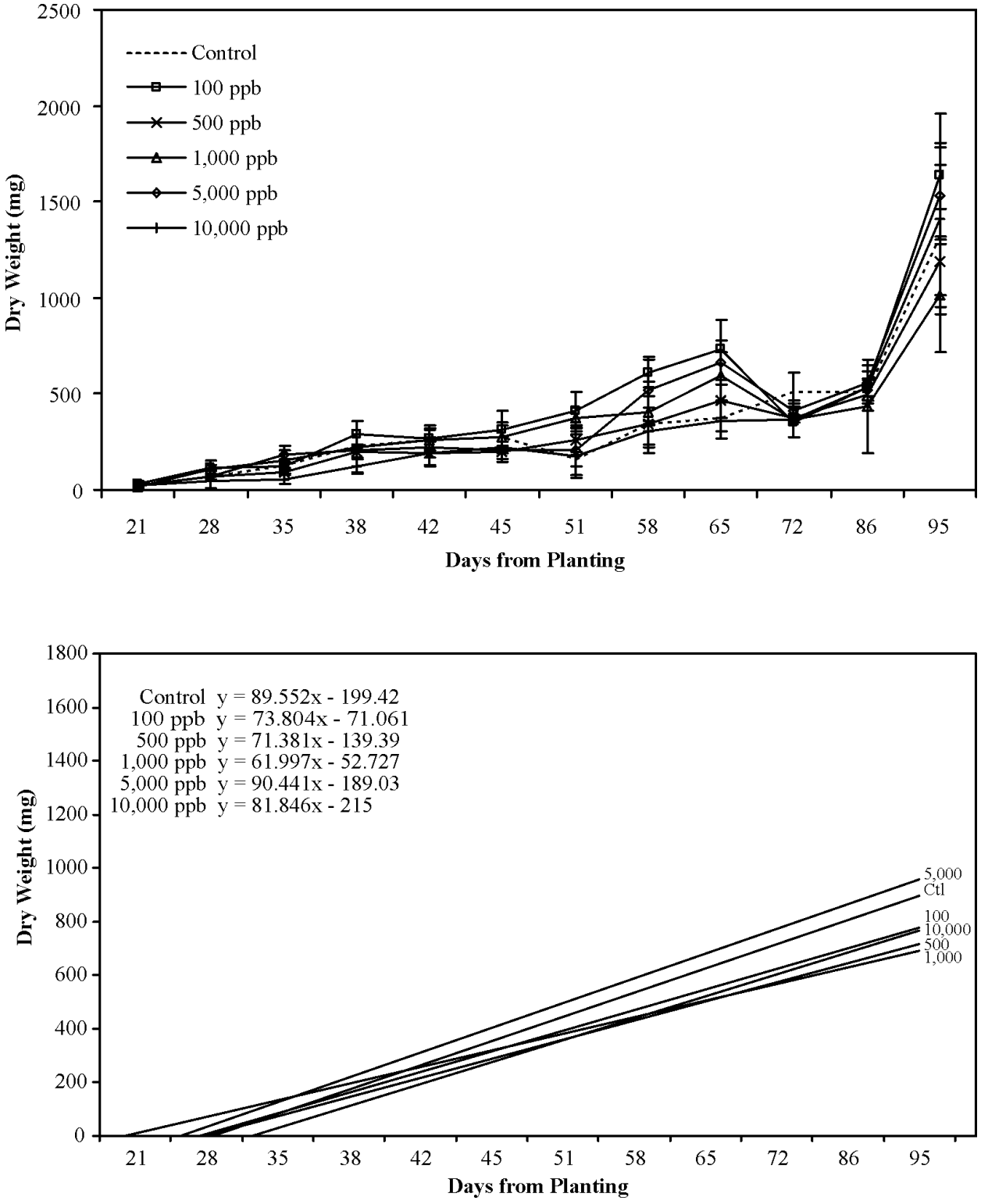

Figure 1. Lettuce dry weights over time. Top graph shows dry mass over time, bottom graph shows dry weight linear regression lines (n=3). The plants were repotted on day-51.

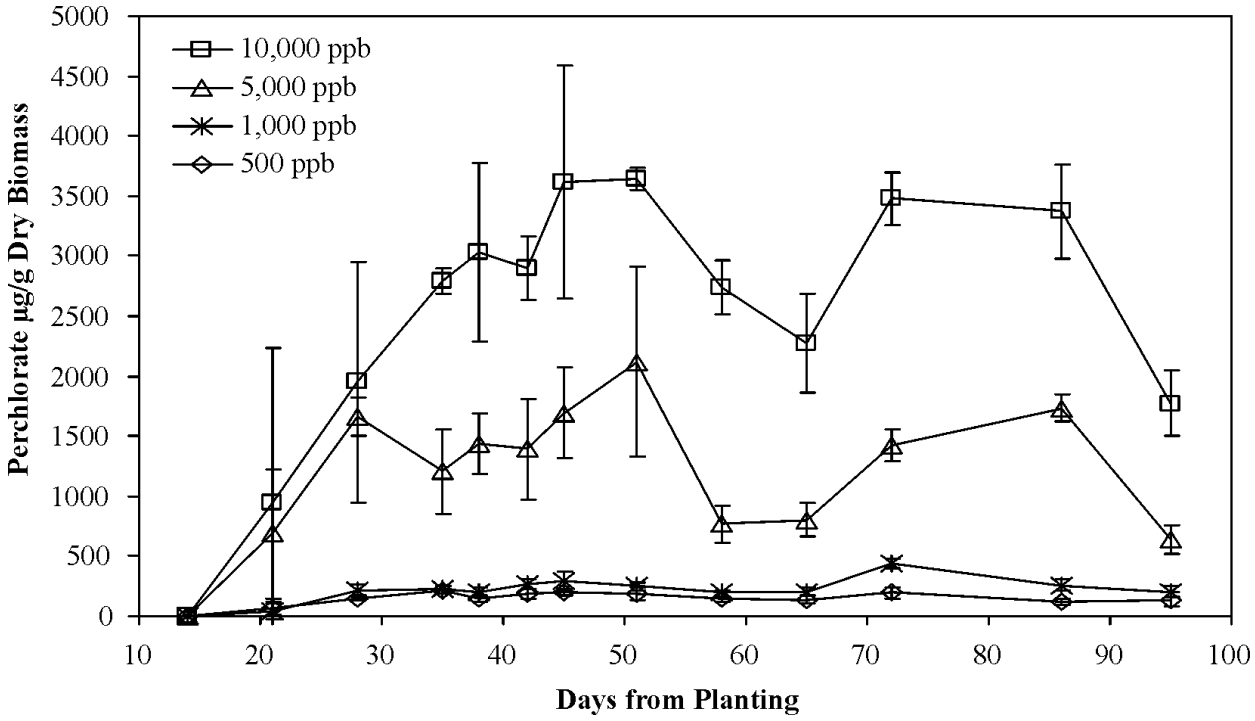

Figure 2. Concentration of perchlorate in dry leaf tissue over time. Error bars represent standard deviation among triplicate samples (n=3).

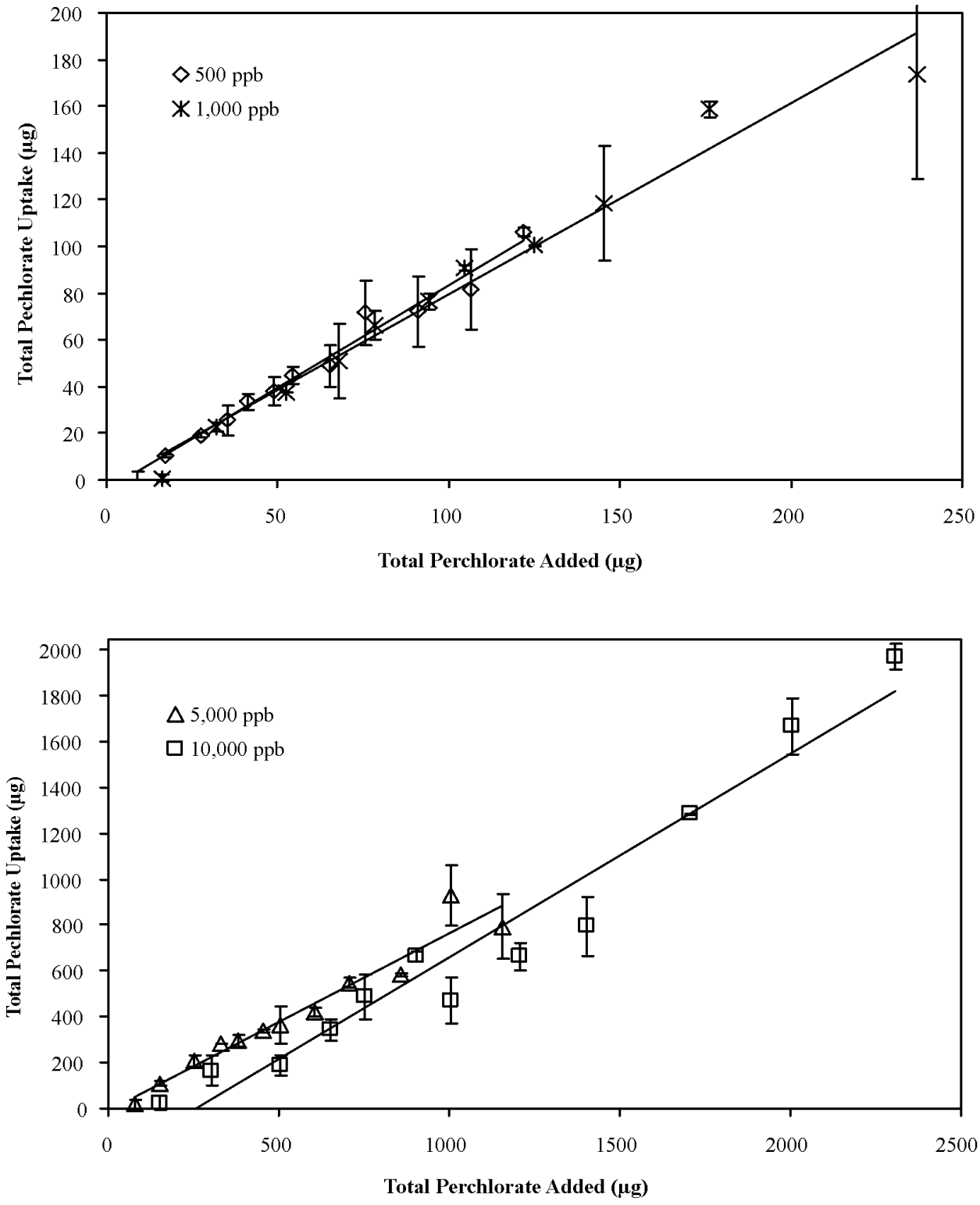

Figure 3. Mass of perchlorate extracted from above-ground lettuce tissue as compared to the mass of perchlorate added.

Table 1. Cumulative volume of perchlorate-fortified water, total mass of perchlorate added, and total mass of perchlorate recovered at each sample point (n = 3). Data is also shown in Figure 2.

		Control			100 ppb Treatment				500 ppb Treatment			
		μg ClO_4^-			μg ClO_4^-				μg ClO_4^-			
Volume of contaminated water	Days from planting	Added in nutrient solution	Recovered	Std Dev	Added as treatment	Total added w/control	Recovered	Std Dev	Added as treatment	Total added w/control	Recovered	Std Dev
15	21	1.5	ND	—	1.5	3.0	TR	0.5	7.5	9.0	2	1.7
30	28	2.0	ND	—	3.0	5.0	2	0.4	15.0	17.0	11	0.5
50	35	2.5	3.8	0.0	5.0	7.5	2	0.0	25.0	27.5	19	0.9
65	38	3.0	5.5	1.6	6.5	9.5	10	1.1	32.5	35.5	26	6.2
75	42	3.5	ND	—	7.5	11.0	10	1.6	37.5	41.0	34	3.3
90	45	4.0	2.0	2.8	9.0	13.0	13	2.8	45.0	49.0	38	6.3
100	51	4.5	3.7	0.4	10.0	14.5	14	1.0	50.0	54.5	45	3.7
120	58	5.0	6.5	0.2	12.0	17.0	16	1.0	60.0	65.0	49	8.9
140	65	5.5	5.9	3.4	14.0	19.5	24	1.2	70.0	75.5	72	14
170	72	6.0	13.0	0.8	17.0	23.0	30	1.3	85.0	91.0	72	15
200	86	6.5	18.0	6.5	20.0	26.5	16	1.5	100.0	106.5	82	17
230	95	7.0	28.0	7.6	23.0	30.0	37	3.5	115.0	122.0	110	1.8

ND = not detected
TR = Trace

		1,000 ppb Treatment				5,000 ppb Treatment				10,000 ppb Treatment			
		μg ClO_4^-				μg ClO_4^-				μg ClO_4^-			
Volume of contaminated water added	Days from planting	Added as treatment	Total added w/control	Recovered	Std Dev	Added as treatment	Total added w/control	Recovered	Std Dev	Added as treatment	Total added w/control	Recovered	Std Dev
15	21	15.0	16.5	0.9	1.5	75.0	76.5	18	22	150.0	151.5	24	30
30	28	30.0	32.0	23.	1.8	150.0	152.0	110	11	300.0	302.0	160	66
50	35	50.0	52.5	37	0.1	250.0	252.5	210	25	500.0	502.5	190	41
65	38	65.0	68.0	51	16	325.0	328.0	280	1.0	650.0	653.0	340	46
75	42	75.0	78.5	66	6.1	375.0	378.5	300	27	750.0	753.5	490	95
90	45	90.0	94.0	76	3.2	450.0	454.0	340	6.4	900.0	904.0	660	21
100	51	100.0	104.5	91	1.3	500.0	504.5	360	83	1000.0	1004.5	470	100
120	58	120.0	125.0	100	0.4	600.0	605.0	420	17	1200.0	1205.0	660	60
140	65	140.0	145.5	120	25	700.0	705.5	550	20	1400.0	1405.5	800	130
170	72	170.0	176.0	160	3.4	850.0	856.0	580	7.2	1700.0	1706.0	1300	3.6
200	86	200.0	206.5	110	8.3	1000.0	1006.5	930	130	2000.0	2006.5	1700	120
230	95	230.0	237.0	170	45	1150.0	1157.0	790	140	2300.0	2307.0	2000	55

Table 2. The mass of perchlorate recovered from each treatment based on best-fit linear regression lines through the entire data set (days 14-95, Figure 3). Coefficient of determination is included for comparison to the significant values of r^2 at p=0.01 (r^2_{sig} = 0.708) for n=10 error degrees of freedom.

Treatment (ppb)	Percent Recovered	r^2
500	82	0.977
1,000	74	0.892
5,000	76	0.944
10,000	73	0.914

Table 3. The mass of perchlorate recovered from each treatment at the final takedown (day 95) based on the average data for the takedown period (n = 3).

Treatment	Perchlorate Added (µg)	Perchlorate Recovered (µg)	Percent Recovered
500	122	110 ± 1.8	90 ± 2
1,000	237	170 ± 45	72 ± 19
5,000	1157	790 ± 140	68 ± 12
10,000	2307	2000 ± 55	87 ± 2

Table 4. Wet plant concentrations, mass of perchlorate recovered from plant tissue, and the resulting concentration factor for the outer leaves and inner head of lettuce at the final takedown (day-95, n = 3).

	Treatment Level											
	500 ppb			1,000 ppb			5,000 ppb			10,000 ppb		
	Wet Plant	Std Dev	CF.*	Wet Plant	Std Dev	CF.*	Wet Plant	Std Dev	CF.*	Wet Plant	Std Dev	CF.*
	ppb			ppb	.		ppb			ppb		
Outer Leaves	14,000	4000	28	21,000	2000	21	84,000	23,000	17	210,000	29,000	21
Inner Leaves (head)	3,000	1000	6	9,000	6000	9	16,000	4,000	3	32,000	4,000	3

* The concentration factor (CF) is the ratio of the wet plant concentration to the treatment concentration.