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Perchlorate Uptake in Spinach As Related to Perchlorate, Nitrate, And Chloride Concentrations in Irrigation Water

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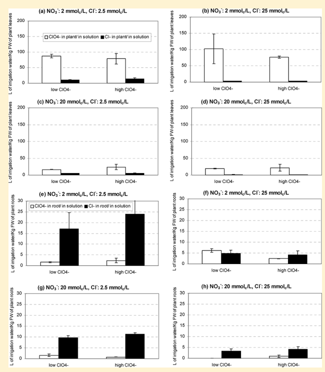
ABSTRACT: Several studies have reported on the detection of perchlorate (ClO_4^-) in edible leafy vegetables irrigated with Colorado River water. However, there is no information on spinach as related to ClO_4^- in irrigation water nor on the effect of other anions on ClO_4^- uptake. A greenhouse ClO_4^- uptake experiment using spinach was conducted to investigate the impact of presence of chloride (Cl^-) and nitrate (NO_3^-) on ClO_4^- uptake under controlled conditions. We examined three concentrations of ClO_4^-, 40, 220, and 400 nmol$_c$/L (nanomoles of charge per liter of solution), three concentrations of Cl^-, 2.5, 13.75, and 25 mmol$_c$/L, and NO_3^- at 2, 11, and 20 mmol$_c$/L. The results revealed that ClO_4^- was taken up the most when NO_3^- and Cl^- were lowest in concentration in irrigation water. More ClO_4^- was detected in spinach leaves than that in the root tissue. Relative to lettuces, spinach accumulated more ClO_4^- in the plant tissue. Perchlorate was accumulated in spinach leaves more than reported for outer leaves of lettuce at 40 nmol$_c$/L of ClO_4^- in irrigation water. The results also provided evidence that spinach selectively took up ClO_4^- relative to Cl^-. We developed a predictive model to describe the ClO_4^- concentration in spinach as related to the Cl^-, NO_3^-, and ClO_4^- concentration in irrigation water.



INTRODUCTION

Perchlorate (ClO_4^-) salt is used as an oxidizing agent in rocket propellants and explosives.[1] Perchlorate has been detected in various water sources, both surface and groundwater, as well as in wine, beverages, baby formula, breast milk, and leafy vegetables.[2−6] Perchlorate has been found in ground and surface water in 35 states in the U.S. Currently, a drinking water standard for ClO_4^- has been set by U.S. Environmental Protection Agency[7] and a few states have established advisory levels (for example, 5 μg/L in New York, 6 μg/L as a maximum contaminant level or public health goal in California, and 14 μg/L in Arizona, ref 8 and 9).

Perchlorate salts are very soluble in water. Once dissolved, the ClO_4^- anion is chemically very stable having a +7 oxidation state and persisting in the environment because of high activation energy necessary for reduction.[10] The main health concern for ClO_4^- ion is that it substitutes iodine (similar charge and ionic radius) and thus interrupts thyroid iodine uptake in human beings[11] resulting in subsequent hormone disruption and potential perturbations of metabolic activities.[12] Perchlorate in water is of concern due to impact on ecosystems[13,14] and an additional pathway for humans' intake via accumulation in vegetables from irrigation water.[15−17]

Elevated concentrations of ClO_4^- have been detected in various groundwater sources, related to the release of ammonium ClO_4^- by military operations, the aerospace industry, and among others. Perchlorate in Colorado River water has been related to ClO_4^- contamination by the ClO_4^- salt manufacturing plant previously located near the Las Vegas wash in Nevada.[10,18−22] The fresh vegetable industry relies on Colorado River water for irrigation in the lower Colorado River regions of California and Arizona. Use of Colorado River water thus caused elevated ClO_4^- concentrations in vegetables.[20,23] More recently, installation of a treatment plant on Las Vegas wash has subsequently reduced the ClO_4^- concentration of the Colorado River.[24]

Spinach has above-ground parts consumed by humans; which makes it a good choice to study ClO_4^- uptake. The interaction between salts and ClO_4^- in edible plants when ClO_4^- is taken up by plant is not fully investigated. Leaf chloride (Cl^-) declined from 4.37 to 2.43% Cl^- as NO_3^- increased from 3 to 15 mmol$_c$/L in wheat (*Triticum aestivum*) leaves.[25] Net uptake rate of NO_3^- in *Plantago maritima* L. was reduced by 23, 33, and 51% at 50, 100, and 200 mol/m^3 NaCl, respectively.[26] Tan and others[14] reported that the uptake of ClO_4^- in smartweed (*Polygonum* spp.) was not greatly affected by the presence of NO_3^--N, SO_4^{2-}, PO_4^{3-}, or Cl^- in 500 mg L^{-1} solution. However, ClO_4^- uptake in three different types of lettuce as independently affected by NO_3^-, SO_4^{2-}, Cl^-, pH, and HCO_3^- was evaluated by Seyfferth et al.[27] They concluded that increasing solution NO_3^- markedly decreased ClO_4^- uptake but observed no severe effect of Cl^- on ClO_4^- uptake in lettuce leaves.

The combined effect of NO_3^- and Cl^- ions on ClO_4^- uptake has not been examined although both NO_3^- and Cl^- are present at varying concentrations in the soil−water during the crop growing season. Also, the accumulation pattern of ClO_4^- in root

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tissues has not been thoroughly investigated under the presence of Cl^- and NO_3^- salts in irrigation water. To date, the research focus has been on lettuce and there are almost no data on ClO_4^- accumulation of other leafy vegetables such as spinach, as related to ClO_4^- concentration in irrigation water, and no information on the effects of ions that may potentially inhibit ClO_4^- uptake in these other leafy vegetables.

The objectives of this study are (1) investigate the uptake of ClO_4^- by spinach as related to ClO_4^- in irrigation water, (2) evaluate the effect of NO_3^- and Cl^- anions on the uptake of ClO_4^- by spinach, (3) examine the physiological effect of ClO_4^- uptake by measuring ClO_4^- concentration in both leaf and root parts and determine the pattern of translocation of ClO_4^- within plant materials, and (4) develop predictive equations to represent ClO_4^- uptake in spinach as related to ClO_4^-, NO_3^-, and Cl^- in irrigation water.

MATERIALS AND METHODS

1. Greenhouse Experiment. The experiment was conducted in 30 sand tanks at the greenhouse facility in U.S. Salinity Laboratory, Riverside, CA. Washed sand (average bulk density: 1.4 Mg/m^3) was contained in sand tanks (1.2 × 0.6 × 0.5 m depth each). After filling up the water reservoir with deionized (DI) water, the water was circulated through the sand several times to ensure equilibration of the saturation within the sand media. The electric conductivity (EC) was monitored before initiating irrigation to ensure low EC in the DI water. Sorption of ClO_4^- onto the surface of the sand particles and container was determined to be negligible and thus is not considered further.

There were 10 different combinations of irrigation water treatments. Each treatment was replicated three times (Table 1). Three randomly selected sand tanks were irrigated with each water composition during the experiment. We utilized three different concentrations of ClO_4^-, 40, 220, and 400 $nmol_c/L$, (nanomoles of charge per liter of solution; approximately 4, 22, and 40 $\mu g/L$, respectively), Cl^- at 2.5, 13.75, and 25 $mmol_c/L$, and NO_3^- at 2, 11, and 20 $mmol_c/L$. Half Hoagland's solution (plant nutrient solution) was prepared and the concentration in the irrigation reservoir in $mmol_c/L$ was: 0.17 KH_2PO_4, 0.75 $MgSO_4 \cdot 7H_2O$, 2.0 KNO_3, 0.25 $CaSO_4$. We utilized DI water and prepared the solutions with reagent grade salts. The pH of the irrigation water ranged between 7.7 and 8.5. The experiment was designed as a classic 2^3 factorial design, with a center point and one additional nonstandard point set to high ClO_4^-, medium NO_3^-, and medium Cl^- level. Seeds of hybrid spinach (*Spinacia oleracea* L., cv. "Space") were purchased from Johnny's seeds (Winslow, ME) and planted in November 2007.

The plants were irrigated in the sand tanks twice a day at 0900 and 1300 h, saturating the sand, and ensuring a uniform root zone solution composition. Irrigation time was 45 min per event. After each irrigation event, the nutrient solution drained back into the 890 L reservoirs below the sand tanks for a subsequent reuse. The ion concentrations in the reservoirs were constantly maintained by supplementing the nutrients every other week, back to the initial nutrient levels. Water loss by evapotranspiration (ET) was replenished by adding DI water back to the reservoirs to maintain constant volumes and osmotic potentials in each reservoir. Spinach was grown for 71 days under controlled greenhouse conditions which are 41% of relative humidity and 18 and 15 °C of day and nighttime temperatures, respectively. Spinach leaves and root tissue samples were harvested from each sand tank at the end of the experiment.

Table 1. Initial Concentrations of ClO_4^- ($nmol_c/L$), Cl^- ($mmol_c/L$), and NO_3^- ($mmol_c/L$) in Irrigation Water

reservoir number	ClO_4^-	Cl^-	NO_3^-	number of replications
1	40	2.5	2	3
2	400	2.5	2	3
3	40	25	2	3
4	400	25	2	3
5	40	2.5	20	3
6	400	2.5	20	3
7	40	25	20	3
8	400	25	20	3
9	220	13.75	11	3
10	400	13.75	11	3

2. Plant Tissue Processing and Perchlorate Extraction. The plant tissue extraction procedure from Seyfferth and Parker[28] was utilized, but weight of plant and volume of water added were modified. The leaves were frozen right after harvesting and stored in the freezer. Approximately 25.0 g of frozen plant sample was weighed and 80.0 mL of DI water was added to plant material for grinding. All standard solutions were made with at least 17.8 MΩ water. After grinding samples, plant material was transferred in 250.0 mL HDPE Nalgene bottle (Nalge Nunc International, Rochester, NY) for 4 h of shaking to release any remaining ClO_4^- to solution. Samples were centrifuged at 5400 RCF (relative centrifugal force) for 1 h. We filtered approximately 30.0 mL of supernatant using 0.2 μm cellulose NO_3^- membrane filters (Whatman International Ltd., Maidstone, England). We took approximately 3.0 mL of filtered aliquot and passed it through a preconditioned ENVI-18 SPE cartridge, discarding the first 1.0 mL of sample and collecting 2.0 mL of liquid sample in the glass tube for ClO_4^- analysis. Perchlorate standard solutions were made from reagent grade sodium perchlorate ($NaClO_4$, Aldrich Chemical Co., Inc., Milwaukee, WI) having density of 2.0 g/cm^3 and molecular weight of 122.44 g/mol.

3. ANALYSIS

3.1. Perchlorate Analysis of Plant Samples and Irrigation Water. Perchlorate was analyzed using an Agilent 1100 series high performance liquid chromatography/mass spectrometry (HPLC/MS). Detailed method for ClO_4^- analysis utilized for this analysis can be obtained by Snyder et al.[29] and U.S. EPA Method 6850. The HPLC settings are briefly discussed as follows. Analytical column: M.IX.MSD1 for LC/MS by Metrohm-Peak; autosampler injection volume: 10.0 μL; HPLC pump—flow rate: 0.7 mL/min, mobile phase: 30% of 50.0 mM ammonium formate (NH_4COOH), and 25 mM ammonium carbonate ($(NH_4)_2CO_3$) mixture +70% of acetonitrile (CH_3CN). These parameter combinations resulted in elution of the perchorate in approximately 16 min, with a total run time of 18 min. Mass spectrometer parameters are, ionization mode: Electrospray (API-ES); polarity: Negative; Spray chamber—drying gas flow: 12.0 L/min, nebulizer pressure: 35 psig, drying gas temperature: 250 °C; SIM parameters—SIM ion: 99.0, fragmentor: 70 V, gain: 1.0 EMV, dwell time: 290 ms, % relative dwell: 100.0; capillary voltage; 3500 Vcap. The response variable of interest in this study is the concentration of ClO_4^- in the fresh weight plant tissue samples (μg/kg FW). The method detection limit (MDL) of HPLC/MS for ClO_4^- was determined to be 0.5 μg/L in plant extract, which was equivalent to 1.6 μg of ClO_4^-/kg FW of plant tissue. As shown in Table 2, some of spinach root samples were below the 1.6 μg/kg of detection limit (left-censored in the statistical analyses). All ClO_4^- concentrations in spinach leaves were above the detection limit.

3.2. Nitrate and Chloride Analysis of Plant Samples and Irrigation Water. The filtered samples after centrifugation (approximately 30.0 mL) were utilized for NO_3^- and Cl^- analysis of plant extracts. Nitrate in plant slurry was measured by UV spectrometry method[30] and Cl^- was determined by coulometric-amperometric titration method.[31]

4. Statistical Methodology. The following statistical analysis was conducted to examine the factors controlling ClO_4^- uptake in spinach and to develop equations relating ClO_4^-, NO_3^-, and Cl^- concentrations in irrigation water to ClO_4^- plant tissue concentration. The following linear factorial model (with 2-way interaction) was fit to both the natural log transformed leaf and root tissue data:

$$\begin{aligned}\ln(\mathrm{ClO_4^-} : \mathrm{accum}) = \beta_0 &+ \beta_1 \ln(\mathrm{ClO_4^-}) \\ &+ \beta_2 \ln(\mathrm{NO_3^-}) + \beta_3 \ln(\mathrm{Cl^-}) \\ &+ \beta_{12} \ln(\mathrm{ClO_4^-}) \times \ln(NO_3^-) \\ &+ \beta_{13} \ln(\mathrm{ClO_4^-}) \times \ln(\mathrm{Cl^-}) \\ &+ \beta_{23} \ln(\mathrm{NO_3^-}) \times \ln(\mathrm{Cl^-}) + \varepsilon \qquad (1)\end{aligned}$$

In eq 1, the ε error term represents an independently, identically and normally distributed error component and the various β parameters quantify the primary (first order) and two-way interaction terms.[32] Positive parameter estimates in this model imply that the log ClO_4^- concentrations in the plant tissue increase as the log transformed ClO_4^-, NO_3^-, and/or Cl^- water concentrations increase, while negative estimates imply that the log ClO_4^- concentrations decrease as these water concentration levels increase.

For the spinach leaves data (where all samples were above the detection limit and thus no censoring occurred), eq 1 was estimated using standard linear modeling techniques.[32] For the left-censored spinach root data, eq 1 was estimated using maximum likelihood techniques.[33] All model estimation was performed using the GLM and LIFETEST procedures in SAS.[34]

Based on the p-values associated with the estimated parameters (Table 3), reduced forms of eq 1 were also fit to each plant tissue data set. These reduced models were estimated by removing all nonsignificant parameter estimates from the linear factorial model (at the 0.05 significance level). Goodness-of-fit (GOF) tests were calculated to assess the adequacy of each fitted equation. For the complete (i.e., noncensored) leaves data sets, traditional lack-of-fit (LOF) F-tests were computed.[32,35] For the left-censored root data sets, asymptotic GOF tests were computed by calculating the log-likelihood (LL) score differences between the reduced and saturated models and then comparing these −2 LL scores to Chi-square distributions with the appropriate degrees of freedom.

The primary goal in each analysis was to identify a parsimonious linear factorial model that fully described how the changing irrigation water ClO_4^-, NO_3^-, and Cl^- concentrations influenced the plant tissue ClO_4^- concentrations.

Table 2. Number of Left-Censored Spinach Root Tissue for Perchlorate Measurements (I.E., Measurements Below the 1.6μg/kg FW Method Detection Limit of ClO_4^-)

treatment[a]	spinach roots
low ClO_4^-, low NO_3^-, low Cl^-	0
high ClO_4^-, low NO_3^-, low Cl^-	0
low ClO_4^-, low NO_3^-, high Cl^-	1
high ClO_4^-, low NO_3^-, high Cl^-	0
low ClO_4^-, high NO_3^-, low Cl^-	1
high ClO_4^-, high NO_3^-, low Cl^-	0
low ClO_4^-, high NO_3^-, high Cl^-	3
high ClO_4^-, high NO_3^-, high Cl^-	0
mid ClO_4^-, mid NO_3^-, mid Cl^-	0
high ClO_4^-, mid NO_3^-, mid Cl^-	0

[a] Low, mid, and high ClO_4^- [ppb] represent 4, 22, and 40, respectively; Low, mid, and high NO_3^- [mmol$_c$/L] represent 2, 11, and 20, respectively.; Low, mid, and high Cl^- [mmol$_c$/L] represent 2.5, 13.75, and 25, respectively.

RESULTS AND DISCUSSION

The sand tank environment was hypothesized to potentially cause ClO_4^- degradation by bacteria in the root zone.[14] Because of this consideration, various researchers utilized an aerated hydroponic system for the laboratory scale plant uptake experiment to minimize the rhizosphere degradation effect on ClO_4^- uptake (refs 14,27,36, etc.). However, commercial leafy vegetables have been grown primarily in soil under field environments. Our experiment was designed to evaluate the combined effect of NO_3^- and Cl^- on ClO_4^- uptake in spinach in a controlled sand tank environment which both more closely reflects field conditions and yet enables accurate monitoring of root zone ClO_4^- concentrations. The concentration of ClO_4^- in reservoirs was monitored and maintained at the constant concentrations (4, 22, and 40 μg/L) throughout the experiment as indicated in the Materials and Methods section. We found no evidence of decrease in ClO_4^- related to a soil process, suggesting that, as expected, our rhizosphere was highly aerobic and ClO_4^- degradation did not need to be further considered in our experiment. ET losses were approximately equal within the range of 1.9 and 2.8 cm of water for all treatments. Low Cl^- treatments had ET loss of 2.23 (average of four treatments) and high Cl^- reservoirs showed 2.55 cm of water, while water losses by ET in low and high NO_3^- treatment reservoirs were 2.38 and 2.4 cm of water, respectively.

The interactive effects of the three independent variables on ClO_4^- in plant parts can best be evaluated by a multivariate

Table 3. Summary Statistics of RMSE and Parameter *P*-Values: Full Factorial Models

data set	RMSE	type III p-values for individual parameter estimates					
		β_1	β_2	β_3	β_{12}	β_{13}	β_{23}
spinach: leaves	0.373	<0.001	0.002	0.154	0.899	0.327	0.402
spinach: roots	0.710	0.235	0.027	0.087	0.170	0.046	0.377

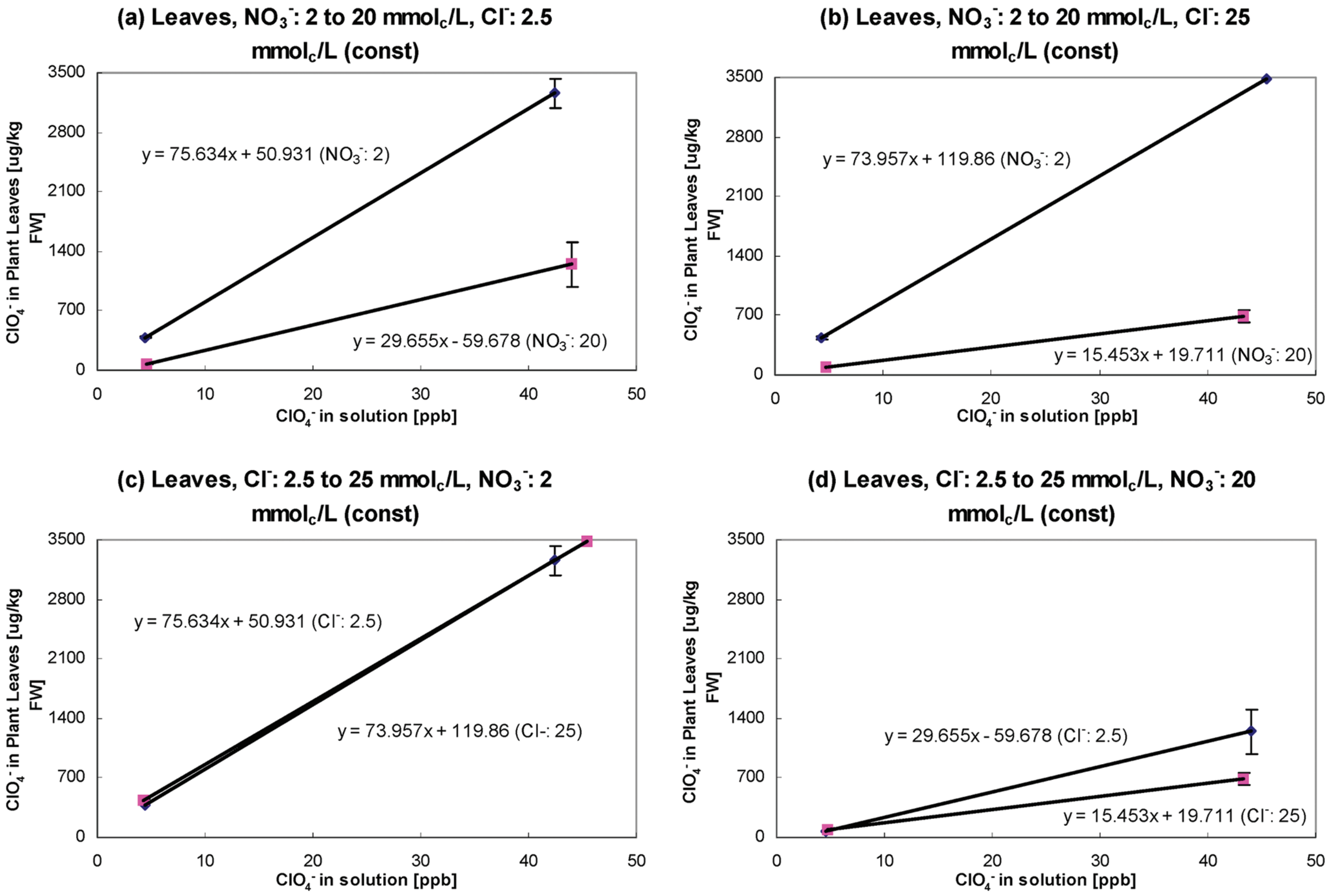

Figure 1. Perchlorate content in spinach leaves as related to irrigation water nitrate and chloride at two perchlorate concentrations. Parts a and b represent the perchlorate content when the chloride concentration is constant at 2.5 and 25 $mmol_c$ /L, respectively. Parts c and d show the perchlorate content when nitrate concentration is constant at 2 and 20 $mmol_c$ /L, respectively. The error bars indicate one standard deviation of the mean where $n = 3$.

statistical analysis. However, for understanding the impact of the various variables it appears useful to examine a subset of the data consisting of end member concentrations of ClO_4^- while holding the other two variables constant, for both high and low concentrations of NO_3^- and Cl^-.

1. Perchlorate Uptake As Related to NO_3^- and Cl^-. Perchlorate content in spinach leaves was dramatically greater than literature reports for iceberg and butterhead lettuce leaves,[27] and appeared to be the highest vegetable ClO_4^- accumulator. However, ClO_4^- in spinach leaves was lower than that for the forage crop alfalfa, grown in sand.[23] As shown in Figure 1, the mean concentration of 3.20 mg/kg of ClO_4^- was obtained when NO_3^- and Cl^- was low. As expected, ClO_4^- in the leaves increased with increased ClO_4^- in solution. Increased NO_3^- in irrigation water suppressed ClO_4^- uptake under both high and low Cl^- (Figure 1a and Figure 1b, respectively). Increased Cl^- had no effect on ClO_4^- uptake under low NO_3^-, as shown in Figure 1c, and only a small reduction under high NO_3^- (Figure 1d) was observed. Spinach appeared to have a different ClO_4^- uptake mechanism compared to iceberg and butterhead lettuce. The highest ClO_4^- concentrations in spinach leaves were obtained when the NO_3^- level was low (2 $mmol_c$/L). Also, Cl^- had a smaller effect on ClO_4^- uptake in spinach as compared to lettuce (Ha and Suarez, in preparation). As shown in Figure 2, the data for spinach roots indicated that ClO_4^- accumulation was greatly suppressed by elevated NO_3^- but only slightly affected by elevated Cl^- in irrigation water.

2. Statistical Analysis. Table 4 showed the pertinent model summary statistics and reduced factorial model parameter estimates for two spinach data sets. The only statistically significant parameter estimates in the reduced factorial model associated with the leaf data were the main $\ln(ClO_4^-)$ and $\ln(NO_3^-)$ effects. The positive $\ln(ClO_4^-)$ parameter estimate implied that increased ClO_4^- concentrations in irrigation water translating into higher ClO_4^- accumulation levels in the leaf tissue. Likewise, the negative $\ln(NO_3^-)$ parameter estimate implied that as the NO_3^- level increased, the ClO_4^- accumulation level in the plant tissue decreased. Interestingly, the $\ln(Cl^-)$ main effect was not statistically significant in this model, suggesting that the $\ln(Cl^-)$ anion levels did not influence ClO_4^- accumulation in spinach leaves.

A somewhat more complex factorial model needed to be employed to adequately describe the $\ln(ClO_4^-)$ accumulation pattern in the spinach root tissue samples. With respect to main effects, the log ClO_4^- accumulation level increased as the $\ln(ClO_4^-)$ anion concentration level increased, and the accumulation level decreased as both the $\ln(NO_3^-)$ and $\ln(Cl^-)$ anion concentration levels increased. Additionally, the positive $\ln(ClO_4^-)$ x $\ln(Cl^-)$ parameter estimate implied that the $\ln(ClO_4^-)$ accumulation rate attributed specifically to the

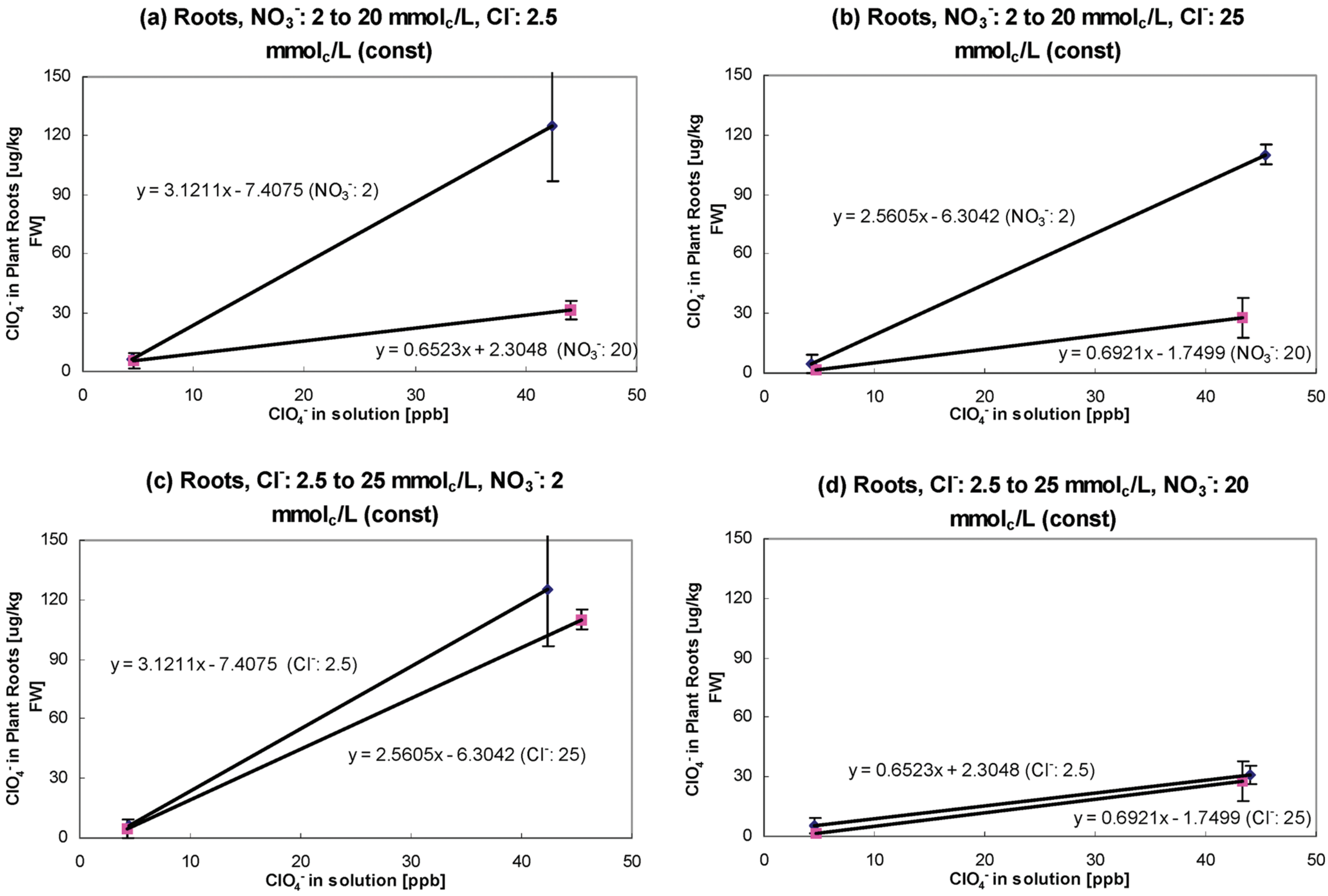

Figure 2. Perchlorate content in spinach roots as related to irrigation water nitrate and chloride at two perchlorate concentrations. Parts a and b represent the perchlorate content when the chloride concentration is constant at 2.5 and 25 mmol$_c$ /L, respectively. Parts c and d show perchlorate content when nitrate concentration is constant at 2 and 20 mmol$_c$ /L, respectively. The error bars indicate one standard deviation of the mean where n = 3.

Table 4. Reduced Factorial Model Summary Statistics and Parameter Estimates (With Associated Standard Errors): Spinach Data Sets[a]

model statistics	leaves	roots
RMSE	0.373	0.725
r (correlation)	0.972	0.814
GOF p-value	0.754	0.276
parameter estimates		
intercept (std.error)	5.107 (0.21)	2.291 (0.79)
$_1$(std.error)	1.034 (0.06)	0.730 (0.26)
$_2$(std.error)	−0.747 (0.06)	−0.596 (0.13)
$_3$ (std.error)	ne	−0.746 (0.34)
$_{12}$(std.error)	ne	ne
$_{13}$(std.error)	ne	0.212 (0.11)
$_{23}$(std.error)	ne	ne

[a] Note: ne = not estimated.

$\ln(ClO_4^-)$ anion concentration level increases as the $\ln(Cl^-)$ concentration level rose.

In the spinach tissue samples, the root-mean-square error (RMSE) estimate (unit: μg/L) for the root model is about two times bigger than the leaf RMSE estimate (0.725 versus 0.373). This result suggests that the relative variation in $\ln(ClO_4^-)$ accumulation in the roots is greater than the relative variation in the leaves. Additionally, both spinach models also exhibit non-significant GOF test statistics. These results indicate that these fitted factorial models adequately describe the leaf and root tissue sample data collected from the spinach crop.

3. Ion Uptake and Translocation. Competition between ions, for the example of NO_3^- and Cl^- during plant uptake process, has been known to be significant for crop production.[37] Competition between NO_3^- and Cl^- on ClO_4^- uptake in higher plants has not still been extensively investigated. In order to compare ion uptake of different anions and evaluate ion specific mechanisms, ratios of concentrations in the plant to the concentrations in irrigation water [bioconcentration factor (BCF)] were calculated. In this instance we calculated ratios of ClO_4^- and Cl^- in the plant leaves and roots to the ion concentrations in irrigation water. The relative uptake of spinach is shown in Figures 3 and 4, which has ratios that are expressed in μg/kg FW divided by μg/L (ppb) for ClO_4^- and g/kg FW divided by g/L for Cl^-. Student's paired t test with two-tailed distribution was conducted with ClO_4^-/ClO_4^- and Cl^-/Cl^- ratio data.

Although it was known that plant roots did not appear to take up ions selectively without having specific transporters for specific ions,[27,37] our results clearly indicated that there was a different uptake pattern of ClO_4^- and Cl^- in roots among the different anion concentrations in irrigation water. Seyfferth et al.[27] also cited Marschner[37] for the statement that Cl^- and

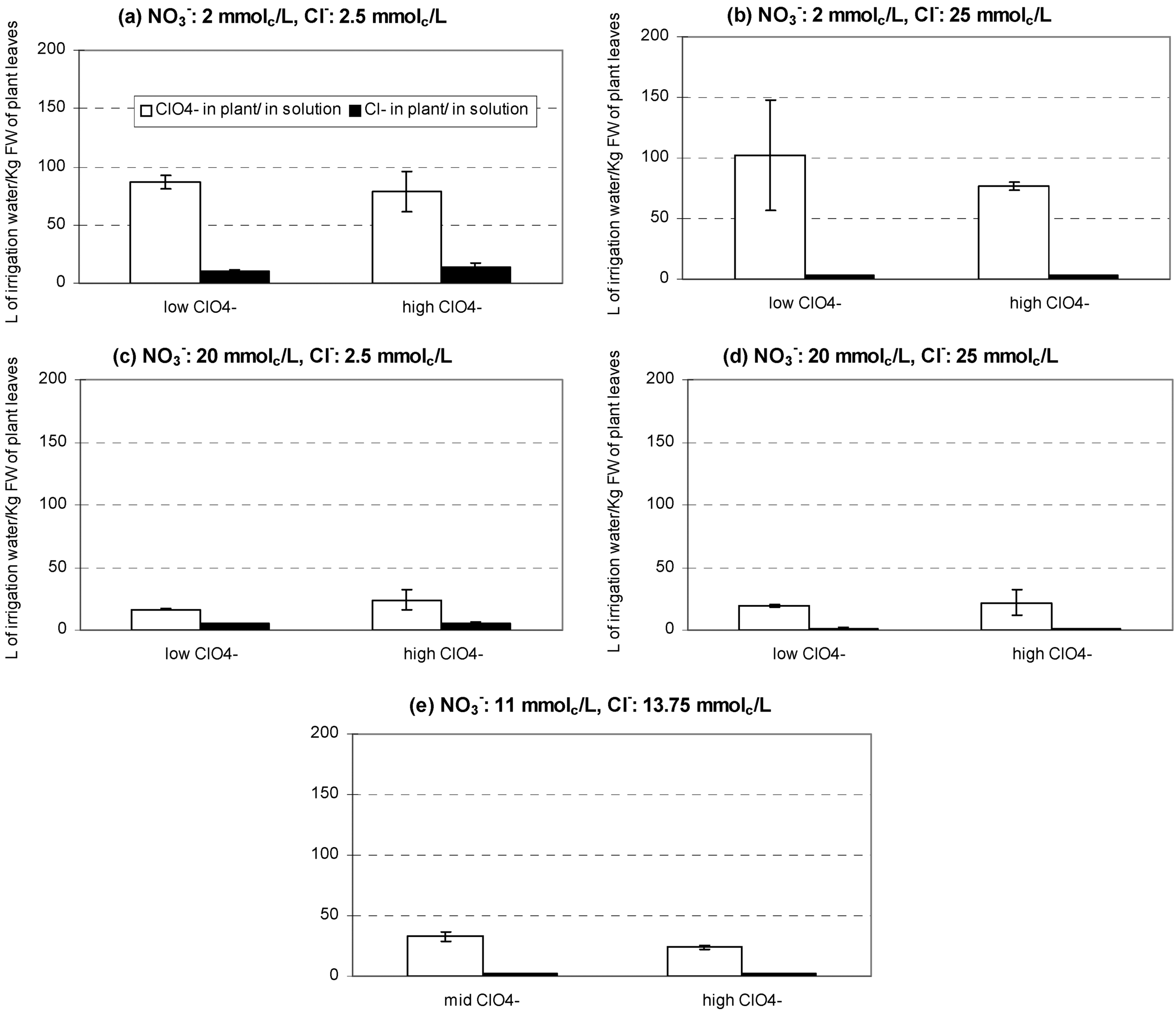

Figure 3. Perchlorate and chloride concentration in spinach leaves divided by concentration in solution. Low, mid, and high indicate ClO_4^- concentrations of 40, 220, and 400 nmol$_c$/L, respectively. Error bars represent one standard deviation of the mean where $n = 3$.

NO_3^- competed each other for Cl^- uptake in Barley plants. They commented that the ClO_4^- uptake mechanism was affected by NO_3^- in plant tissue as a result of sharing a common anion transport mechanism in higher plants. Perchlorate uptake in iceberg and butterhead lettuce was affected by NO_3^-, as reported earlier[27] from their hydrophonic growth chamber system. Based on ref 27 the BCF values for crisp head were calculated approximately between 11.0 and 20.0 with the NO_3^- ranges between 4 and 12 mM, and between 11.6 and 14.0 with the Cl^- ranges between 5 and 15 mM. In contrast, the BCF values in this study ranged from 16.6 shown in Figure 3c at high NO_3^- concentrations to 102.1 shown in Figure 3b at low NO_3^- concentrations. The reported BCF for alfalfa forage crop was approximately 360.[23]

The ratio of leaf/solution concentration of ClO_4^- and Cl^- is presented in Figure 3. These data all showed that ClO_4^- was preferentially accumulated relative to Cl^- under all conditions. Under low Cl^- and NO_3^- concentrations, the accumulation of ClO_4^- relative to solution concentration was about 7 times higher than that for Cl^- (Figure 3a). These data are in contrast to lettuce data, in which the Cl^- concentration ratio is roughly comparable but the ClO_4^- ratio is much lower. Based on the ratio data it is clear that spinach leaves accumulate more ClO_4^- compared to that in lettuce leaves (data not shown).

Increasing solution Cl^- concentration suppressed the spinach leaf/solution Cl^- ratio and resulted in a slight increase in ClO_4^- leaf/solution ratio when ClO_4^- was low and almost no change at higher ClO_4^- (Figure 3b). These data are in contrast to lettuce data where increased Cl^- suppressed the ClO_4^- ratio (data not shown). In contrast to Cl^-, NO_3^- drastically suppressed ClO_4^- ratios (Figure 3c). These data demonstrate that spinach has a different uptake or translocation mechanism for ClO_4^- and increased Cl^- does not affect ClO_4^- accumulation in the leaves. Interestingly, Cl^- uptake in spinach leaves was not high when compared to Cl^- uptake in lettuce leaves (data not shown).

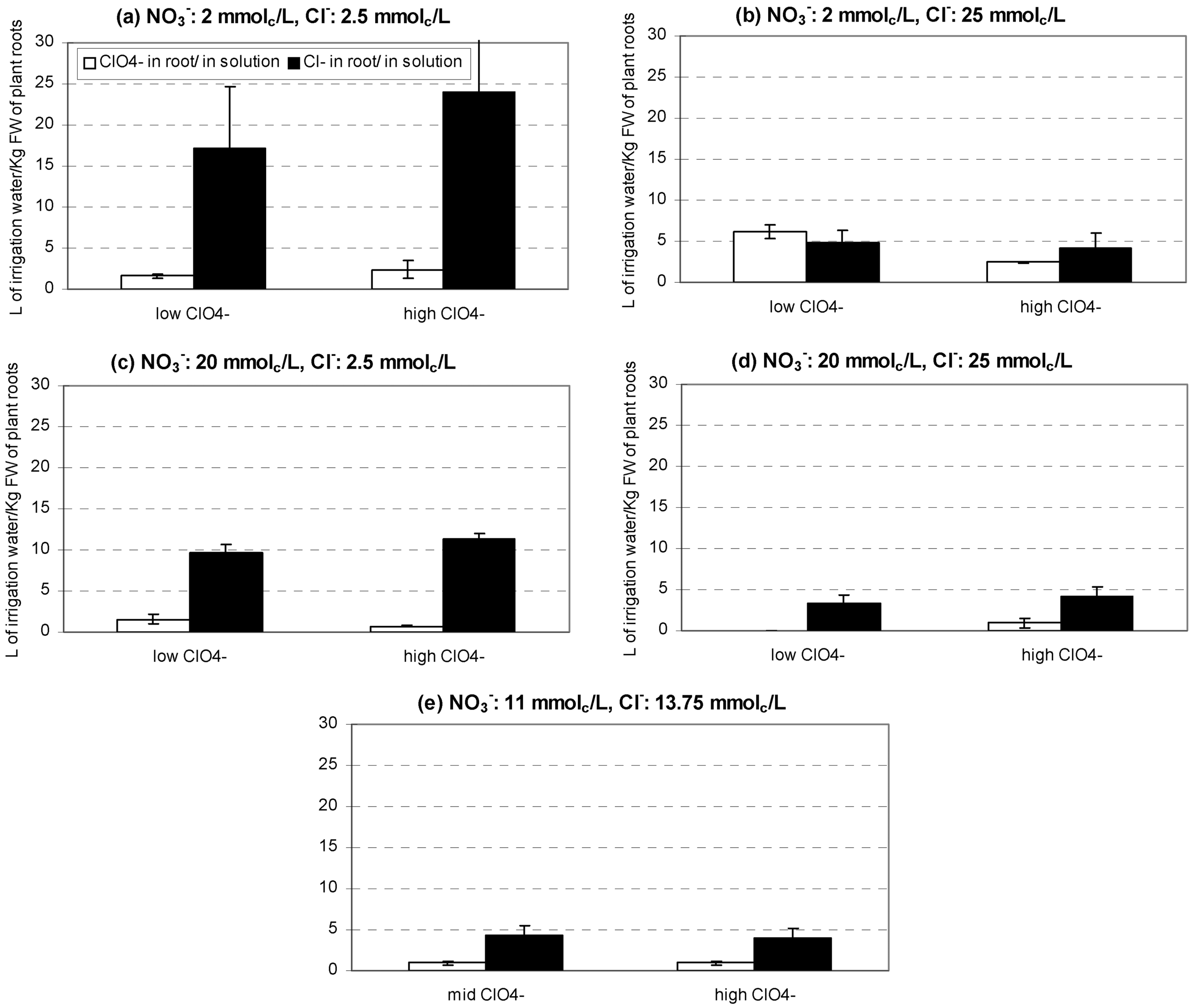

Figure 4. Perchlorate and chloride concentration in spinach roots divided by concentration in solution. Low, mid, and high indicate ClO_4^- concentrations of 40, 220, and 400 nmol$_c$/L, respectively. Error bars represent one standard deviation of the mean where n = 3.

In contrast to the leaf data, spinach roots showed a high root/solution Cl^- ratio and a very low root/solution ClO_4^- ratio (Figure 4a) under low NO_3^- and Cl^- in solution. As Cl^- increased in solution, the Cl^- root/solution ratio decreased as observed in leaves, and the ClO_4^- ratio is increased in low ClO_4^- but similar in magnitude for both high and low ClO_4^- levels (compare Figure 4a and Figure 4b). Increased solution NO_3^- suppressed Cl^- and ClO_4^- uptake in spinach root (compare Figure 4c with Figure 4a). Based on these data it appears that transport of ClO_4^- and Cl^- in the spinach plant is vastly different, with ClO_4^- transport being similar in the leaves and Cl^- transport being restricted to the leaves as Cl^- increases in irrigation water.

Chloride uptake by spinach (*Spinacia oleracea* L.) leaves and roots was earlier investigated by Speer and Kaiser.[38] In their study, spinach was treated in 100 mmol/L NaCl solution in a growth chamber for 10 days. Another set of experiment required a few stepwise increments of 100 mmol/L NaCl solution to reach at the final concentration of 300 mmol/L NaCl in solution to evaluate Na^+ and Cl^- distribution between symplastic and apoplastic space of leaves for 17 days. The results of the first experiment of Speer and Kaiser[38] showed that the Cl^- concentration in spinach leaves reached a relatively low pseudosteady state after the fourth day of the experiment. Slightly less NaCl was accumulated in spinach roots compared to spinach leaves in four days and spinach roots ended up accumulating more NaCl in 10 days. However, the total Cl^- concentration of leaves and roots resulted in means with error bars indicating that the standard deviations were very close to each other. Also, it was noted by Speer and Kaiser[38] that spinach accrued more Cl^- in symplast space of leaves than in apoplasm.

Our experimental results also revealed that there was a small Cl^- uptake ratio reduction in spinach roots when Cl^- concentration is high (25 mmol$_c$/L) when NO_3^- concentration increased (compare Figure 4b with Figure 4d). This phenomenon was also examined by Glass and Siddiqi[39] with their experiment using barley plants. Marschner[37] mentioned Glass and Siddiqi[39]'s work where the inhibition of Cl^- uptake with an increase in

NO_3^- in solution appeared to result from a negative feedback from NO_3^- stored in the vacuoles of root cells and Cl^- influx at the plasma membrane. The Cl^- concentration in barley shoots was greatly reduced in the presence of NO_3^- in solution. Although there were no experimental results of barley roots reported by Glass and Siddiqi,[39] their barley shoot results looked similar to our Cl^- uptake experimental results of spinach leaves.

The following findings were obtained: (1) The $\ln(ClO_4^-)$ leaf accumulation shows a small increase as the $\ln(ClO_4^-)$ anion concentration in irrigation water increases and $\ln(ClO_4^-)$ leaf accumulation decreases as the $\ln(NO_3^-)$ and $\ln(Cl^-)$ anion concentrations increase. (2) There are few statistically significant anion interactions in the leaf $\ln(ClO_4^-)$ accumulation models. In contrast, the root $\ln(ClO_4^-)$ accumulation model is more complex, exhibiting more statistically significant anion interaction parameter estimates, although the main effect trends are consistent across both the root and leaf tissue samples. (3) Transport of ClO_4^- and Cl^- in the spinach plant is largely different between leaves and roots; ClO_4^- translocation is constant in the leaves and Cl^- transport is being restricted to the leaves when Cl^- increases in irrigation water. The mass balance of ClO_4^- was not estimated due to the missing information of total weight of spinach harvest, thus this would be a limitation of this study.

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