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 (ClO4−) in edible leafy vegetables irrigated with Colorado River water. However, there is no information on spinach as related to ClO4− in irrigation water nor on the effect of other anions on ClO4− uptake. A greenhouse ClO4− uptake experiment using spinach was conducted to investigate the impact of presence of chloride (Cl−) and nitrate (NO3−) on ClO4− uptake under controlled conditions. We examined three concentrations of ClO4−, 40, 220, and 400 nmol/L (nanomoles of charge per liter of solution), three concentrations of Cl−, 2.5, 13.75, and 25 mmol/L, and NO3− at 2, 11, and 20 mmol/L. The results revealed that ClO4− was taken up the most when NO3− and Cl− were lowest in concentration in irrigation water. More ClO4− was detected in spinach leaves than that in the root tissue. Relative to lettuces, spinach accumulated more ClO4− in the plant tissue. Perchlorate was accumulated in spinach leaves more than reported for outer leaves of lettuce at 40 nmol/L of ClO4− in irrigation water. The results also provided evidence that spinach selectively took up ClO4− relative to Cl−. We developed a predictive model to describe the ClO4− concentration in spinach as related to the Cl−, NO3−, and ClO4− concentration in irrigation water.

INTRODUCTION

Perchlorate (ClO4−) 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 ClO4− has been set by U.S. Environmental Protection Agency7 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 ClO4− 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 ClO4− 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 ecosystems13,14 and an additional pathway for humans’ intake via accumulation in vegetables from irrigation water.15–17

Elevated concentrations of ClO4− have been detected in various groundwater sources, related to the release of ammonium ClO4− by military operations, the aerospace industry, and among others. Perchlorate in Colorado River water has been related to ClO4− contamination by the ClO4− salt manufacturing plant previously located near the Las Vegas wash in Nevada.18,19–22 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 ClO4− concentrations in vegetables.20,23 More recently, installation of a treatment plant on Las Vegas wash has subsequently reduced the ClO4− concentration of the Colorado River.24

Spinach has above-ground parts consumed by humans; which makes it a good choice to study ClO4− uptake. The interaction between salts and ClO4− in edible plants when ClO4− is taken up by plant is not fully investigated. Leaf chloride (Cl−) declined from 4.37 to 2.43% Cl− as NO3− increased from 3 to 15 mmol/L in wheat (Triticum aestivum) leaves.25 Net uptake rate of NO3− in Plantago maritima L. was reduced by 23, 33, and 51% at 50, 100, and 200 mol/m3 NaCl, respectively.26 Tan and others27 reported that the uptake of ClO4− in smartweed (Polygonum spp.) was not greatly affected by the presence of NO3−, SO42−, PO43−, or Cl− in 500 mg L−1 solution. However, ClO4− uptake in three different types of lettuce as independently affected by NO3−, SO42−, Cl−, pH, and HCO3− was evaluated by Seyfferth et al.27 They concluded that increasing solution NO3− markedly decreased ClO4− uptake but observed no severe effect of Cl− on ClO4− uptake in lettuce leaves.

The combined effect of NO3− and Cl− ions on ClO4− uptake has not been examined although both NO3− and Cl− are present at varying concentrations in the soil—water during the crop growing season. Also, the accumulation pattern of ClO4− in root
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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 $\times$ 0.6 $\times$ 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 treatments. Each treatment was replicated three times (Table 1).

### Table 1. Initial Concentrations of ClO$_4^-$ (nmol/L), Cl$^-$ (nmol/L), and NO$_3^-$ (nmol/L) in Irrigation Water

<table>
<thead>
<tr>
<th>reservoir number</th>
<th>ClO$_4^-$</th>
<th>Cl$^-$</th>
<th>NO$_3^-$</th>
<th>number of replications</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>2.5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>400</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>40</td>
<td>2.5</td>
<td>2</td>
<td>3</td>
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<tr>
<td>4</td>
<td>400</td>
<td>2.5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>2.5</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>400</td>
<td>2.5</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>40</td>
<td>25</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>400</td>
<td>25</td>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>220</td>
<td>13.75</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>400</td>
<td>13.75</td>
<td>11</td>
<td>3</td>
</tr>
</tbody>
</table>

and root tissue samples were harvested from each sand tank at the end of the experiment.

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 $\Omega$ 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 $\mu$m 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 $\mu$L; HPLC pump—flow rate: 0.7 mL/min, mobile phase: 30% of 50.0 mM ammonium formate (NH$_4$COOH), and 25 mM ammonium carbonate ((NH$_4$)$_2$CO$_3$) mixture +70% of acetonitrile (CH$_3$CN). These parameter combinations resulted in elution of the perchlorate 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 $^\circ$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:

$$\ln(\text{ClO}_4^- \text{ : accum}) = \beta_0 + \beta_1 \ln(\text{ClO}_4^-) + \beta_2 \ln(\text{NO}_3^-) + \beta_3 \ln(\text{Cl}^-) + \beta_{12} \ln(\text{ClO}_4^-) \times \ln(\text{NO}_3^-) + \beta_{13} \ln(\text{ClO}_4^-) \times \ln(\text{Cl}^-) + \beta_{23} \ln(\text{NO}_3^-) \times \ln(\text{Cl}^-) + \epsilon \quad (1)$$

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.$^{33}$ 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., uncensored) 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.

## 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 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$_4^-$)

<table>
<thead>
<tr>
<th>treatment*</th>
<th>spinach roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>low ClO$_4^-$, low NO$_3^-$, low Cl$^-$</td>
<td>0</td>
</tr>
<tr>
<td>high ClO$_4^-$, low NO$_3^-$, low Cl$^-$</td>
<td>0</td>
</tr>
<tr>
<td>low ClO$_4^-$, low NO$_3^-$, high Cl$^-$</td>
<td>1</td>
</tr>
<tr>
<td>high ClO$_4^-$, low NO$_3^-$, high Cl$^-$</td>
<td>0</td>
</tr>
<tr>
<td>low ClO$_4^-$, high NO$_3^-$, low Cl$^-$</td>
<td>1</td>
</tr>
<tr>
<td>high ClO$_4^-$, high NO$_3^-$, low Cl$^-$</td>
<td>0</td>
</tr>
<tr>
<td>low ClO$_4^-$, high NO$_3^-$, high Cl$^-$</td>
<td>3</td>
</tr>
<tr>
<td>high ClO$_4^-$, high NO$_3^-$, high Cl$^-$</td>
<td>0</td>
</tr>
<tr>
<td>mid ClO$_4^-$, mid NO$_3^-$, mid Cl$^-$</td>
<td>0</td>
</tr>
<tr>
<td>high ClO$_4^-$, mid NO$_3^-$, mid Cl$^-$</td>
<td>0</td>
</tr>
</tbody>
</table>

*Low, mid, and high ClO$_4^-$ [ppb] represent 4, 22, and 40, respectively; Low, mid, and high NO$_3^-$ [mmol/L] represent 2, 11, and 20, respectively; Low, mid, and high Cl$^-$ [μmol/L] represent 2.5, 13.75, and 25, respectively.

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

<table>
<thead>
<tr>
<th>data set</th>
<th>RMSE</th>
<th>β₁</th>
<th>β₂</th>
<th>β₃</th>
<th>β₁₂</th>
<th>β₁₃</th>
<th>β₂₃</th>
</tr>
</thead>
<tbody>
<tr>
<td>spinach: leaves</td>
<td>0.373</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.154</td>
<td>0.899</td>
<td>0.327</td>
<td>0.402</td>
</tr>
<tr>
<td>spinach: roots</td>
<td>0.710</td>
<td>0.235</td>
<td>0.027</td>
<td>0.087</td>
<td>0.170</td>
<td>0.046</td>
<td>0.377</td>
</tr>
</tbody>
</table>
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, 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. 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 mmolc/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
\[ \ln(\text{ClO}_4^-) \text{ anion concentration level increases as the } \ln(\text{Cl}^-) \text{ concentration level rose.} \]

In the spinach tissue samples, the root-mean-square error (RMSE) estimate (unit: \(\mu\text{g/L}\)) 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 \(\ln(\text{ClO}_4^-)\) 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 \(\text{NO}_3^-\) and \(\text{Cl}^-\) during plant uptake process, has been known to be significant for crop production.\textsuperscript{37} Competition between \(\text{NO}_3^-\) and \(\text{Cl}^-\) on \(\text{ClO}_4^-\) 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 \(\text{ClO}_4^-\) and \(\text{Cl}^-\) 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 \(\mu\text{g/kg FW} \div \mu\text{g/L} \text{ (ppb)}\) for \(\text{ClO}_4^-\) and \(g/kg \text{ FW} \div g/L \text{ for } \text{Cl}^-\). Student’s paired \(t\) test with two-tailed distribution was conducted with \(\text{ClO}_4^- /\text{ClO}_4^-\) and \(\text{Cl}^- /\text{Cl}^-\) ratio data.

Although it was known that plant roots did not appear to take up ions selectively without having specific transporters for specific ions,\textsuperscript{37,37} our results clearly indicated that there was a different uptake pattern of \(\text{ClO}_4^-\) and \(\text{Cl}^-\) in roots among the different anion concentrations in irrigation water. Seyffarth et al.\textsuperscript{37} also cited Marschner\textsuperscript{37} for the statement that \(\text{Cl}^-\) and

Table 4. Reduced Factorial Model Summary Statistics and Parameter Estimates (With Associated Standard Errors): Spinach Data Sets\textsuperscript{a}

<table>
<thead>
<tr>
<th>model statistics</th>
<th>leaves</th>
<th>roots</th>
</tr>
</thead>
<tbody>
<tr>
<td>RMSE</td>
<td>0.373</td>
<td>0.725</td>
</tr>
<tr>
<td>(r) (correlation)</td>
<td>0.972</td>
<td>0.814</td>
</tr>
<tr>
<td>GOF (p)-value</td>
<td>0.754</td>
<td>0.276</td>
</tr>
<tr>
<td>parameter estimates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>intercept (std.error)</td>
<td>5.107 (0.21)</td>
<td>2.291 (0.79)</td>
</tr>
<tr>
<td>1 (std.error)</td>
<td>1.034 (0.06)</td>
<td>0.730 (0.26)</td>
</tr>
<tr>
<td>2 (std.error)</td>
<td>-0.747 (0.06)</td>
<td>-0.596 (0.13)</td>
</tr>
<tr>
<td>3 (std.error)</td>
<td>ne</td>
<td>-0.746 (0.34)</td>
</tr>
<tr>
<td>11 (std.error)</td>
<td>ne</td>
<td>ne</td>
</tr>
<tr>
<td>12 (std.error)</td>
<td>ne</td>
<td>ne</td>
</tr>
<tr>
<td>13 (std.error)</td>
<td>ne</td>
<td>0.212 (0.11)</td>
</tr>
<tr>
<td>23 (std.error)</td>
<td>ne</td>
<td>ne</td>
</tr>
</tbody>
</table>

\(a\) Note: ne = not estimated.

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/L, respectively. Parts c and d show perchlorate content when nitrate concentration is constant at 2 and 20 mmol/L, respectively. The error bars indicate one standard deviation of the mean where \(n = 3\).
NO$_3^-$ competed each other for Cl$^-$/C0 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).
In contrast to the leaf data, spinach roots showed a high root/solution Cl\(^{-}\)/C\(_{0}\) ratio and a very low root/solution ClO\(_{4}\)/C\(_{0}\) ratio (Figure 4a) under low NO\(_{3}\)/C\(_{0}\) and Cl\(^{-}\)/C\(_{0}\) in solution. As Cl\(^{-}\) increased in solution, the Cl\(^{-}\) root/solution ratio decreased as observed in leaves, and the ClO\(_{4}\)/C\(_{0}\) 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\(_{3}\)/C\(_{0}\) suppressed Cl\(^{-}\) and ClO\(_{4}\)/C\(_{0}\) uptake in spinach root (compare Figure 4c with Figure 4a). Based on these data it appears that transport of ClO\(_{4}\)/C\(_{0}\) and Cl\(^{-}\)/C\(_{0}\) 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 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\(^{-}\)/C\(_{0}\) 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 apoplast.

Our experimental results also revealed that there was a small Cl\(^{-}\) uptake ratio reduction in spinach roots when Cl\(^{-}\) concentration is high (25 mmolc/L) when NO\(_{3}\)/C\(_{0}\) 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

Figure 4. Perchlorate and chloride concentration in spinach roots divided by concentration in solution. Low, mid, and high indicate ClO\(_{4}\)/C\(_{0}\) concentrations of 40, 220, and 400 mmolc/L, respectively. Error bars represent one standard deviation of the mean where n = 3.
NO₃⁻ in solution appeared to result from a negative feedback from NO₃⁻ 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 NO₃⁻ 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(ClO₄⁻/) leaf accumulation shows a small increase as the ln(ClO₄⁻/) anion concentration in irrigation water increases and ln(ClO₄⁻/) leaf accumulation decreases as the ln(NO₃⁻/) and ln(Cl⁻/) anion concentrations increase. (2) There are few statistically significant anion interactions in the leaf ln(ClO₄⁻/) accumulation models. In contrast, the root ln(ClO₄⁻/) 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 ClO₄⁻ and Cl⁻ in the spinach plant is largely different between leaves and roots; ClO₄⁻ 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 ClO₄⁻ 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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