

# Accumulation of perchlorate in tobacco plants: development of a plant kinetic model†

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Previous studies have shown that tobacco plants are tolerant of perchlorate and will accumulate perchlorate in plant tissues. This research determined the uptake, translocation, and accumulation of perchlorate in tobacco plants. Three hydroponics growth studies were completed under greenhouse conditions. Depletion of perchlorate in the hydroponics nutrient solution and accumulation of perchlorate in plant tissues were determined at two-day intervals using ion chromatography. Perchlorate primarily accumulated in tobacco leaves, yielding a substantial storage capacity for perchlorate. Mass balance results show that perchlorate degradation was negligible in plants. Tobacco plants were shown to effectively accumulate perchlorate over a wide range of initial concentrations (10 ppb to 100 ppm) from the hydroponics solution. Results suggest that tobacco plants are potential plants for the phytoremediation of perchlorate. A mathematical model was developed to describe the distribution of perchlorate in tobacco plants under rapid growth conditions. The Plant Kinetic (PK) model defined a plant as a set of compartments, described by mass balance differential equations and plant-specific physiological parameters. Data obtained from a separate hydroponics growth study with multiple solution perchlorate concentrations were used to validate predicted root, stem, and leaf concentrations. There was good agreement between model predictions and measured concentrations in the plant. The model, once adequately validated, can be applied to other terrestrial plants and inorganic chemicals currently used for both phytoremediation and ecological risk assessment.

## 1. Aim of investigation

Perchlorate ( $\text{ClO}_4^-$ ) is an inorganic contaminant in soils, groundwater, surface waters, and irrigation waters used for crop production. Perchlorate is found in the environment as part of certain geologic deposits, such as the Chilean nitrate deposits, and as a contaminant resulting from the use of solid salts of ammonium, potassium, or sodium perchlorate in industrial and commercial applications.<sup>1</sup> Perchlorate metal salts can undergo mild to explosive reactions when in the presence of oxidizable substances. This characteristic has led to the use of perchlorate salts as oxidizers in a number of commercial applications such as solid propellants for rockets, missiles, fireworks, explosives, and pyrotechnic formulations.<sup>2</sup>

In the environment, the water solubilities of perchlorate salts differ, ranging from a solubility of  $0.01 \text{ mol L}^{-1}$  for potassium perchlorate to  $8.5 \text{ mol L}^{-1}$  for sodium perchlorate. The

perchlorate anion is very unreactive and persists for many decades under typical groundwater and surface water conditions.<sup>3</sup> The environmental risks posed by perchlorate are currently being assessed by the United States Environmental Protection Agency, which has placed perchlorate on the Contaminant Candidate List<sup>4</sup> and the Unregulated Contaminant Monitoring Rule.<sup>5</sup>

Since 1997, perchlorate has been discovered at various manufacturing sites and in drinking water and well-water supplies in California, Utah, Nevada, and Texas. Perchlorate has been found in California water supplies in the range of 18 to 280 ppb ( $\mu\text{g L}^{-1}$ ), potentially affecting the drinking water supplies of at least 12 million people.<sup>3</sup> The groundwater locations in California have been associated with twelve facilities, which have manufactured or tested solid rocket fuels for the Department of Defense or the National Aeronautics and Space Administration. Perchlorate has also been detected at low levels (5–9 ppb) in the Colorado River<sup>6</sup> and up to 17 ppb in Lake Mead, as a result of release from two ammonium perchlorate manufacturing facilities in Nevada.<sup>3</sup>

A previous report by Ellington *et al.*<sup>7</sup> indicated that perchlorate in a fertilizer that contained the anion as a natural component was taken up by roots and accumulated in leaves of

†Electronic supplementary information (ESI) available: Tables S1 and S2 showing perchlorate content in tobacco plants and solutions and Figs S1 and S2 showing mean nutrient solution utilization rates and average fresh weights for perchlorate-amended plants. See <http://www.rsc.org/suppdata/em/b3/b300570d>

tobacco plants. Samples of chewing tobaccos, cigars, and cigarettes, which represented products from seven tobacco companies, were purchased at retail stores in Athens, Georgia, in November and December 1999. Perchlorate was found in all but one of the products, with the highest perchlorate concentration of  $149.3 \pm 3.0$  ppm ( $\text{mg kg}^{-1}$ ) dry weight being found in a chewing tobacco. Although the leaf curing practices for these products were unknown, it can be assumed that the perchlorate contained in the products originated in the soil or fertilizer, was translocated during the plant growing process, and persisted in the plant leaves over several years of curing and aging processes. This raises questions regarding the presence of perchlorate in other food crops and products being consumed by human and animal populations that were exposed to perchlorate contamination.

Perchlorate has an ionic radius and charge similar to that of iodide. This allows perchlorate to compete with the uptake of iodide into the thyroid, an essential element for proper thyroid function. Public health concerns for perchlorate are based on its ability to cause an iodide deficiency, which can lead to hypothyroidism. Thyroid hormones are very important for normal growth and development in humans.<sup>8</sup>

A number of approaches have been investigated for the removal of perchlorate contamination from water including reverse osmosis, anion exchange, adsorption by activated carbon, and phytoremediation.<sup>9</sup> Phytoremediation employs plants to remove contaminants from polluted soils in a cost-effective and ecologically sound manner. The possibility of successful phytoremediation of perchlorate-contaminated soil depends on the availability of plant varieties with high rates of accumulation and tolerance for perchlorate.<sup>10</sup> Von Burg<sup>11</sup> reported that in terrestrial plant species, perchlorate soil concentrations in the range of 40 to 80 ppm in soil were toxic to plants. It was also shown that potassium perchlorate concentrations as low as 0.55 ppm in soil inhibited growth in ryegrass and cotton. Soybeans were reported to show toxic symptoms when nurtured with water containing 2.5 to 5 ppm ( $\text{mg L}^{-1}$ ) potassium perchlorate.<sup>11</sup>

Previous field and laboratory studies have shown that perchlorate is transported from contaminated soils through the root system and is accumulated in the tissues and organs of vascular plants. Concentrations of perchlorate in tobacco plant tissues and soils have been measured from a 1999 crop grown in soil fertilized with Chilean saltpeter.<sup>7</sup> Perchlorate concentration in the leaf lamina was  $96.0 \pm 0.6$  ppm dry weight ( $14.6 \pm 0.1$  ppm fresh weight). Perchlorate concentrations in tobacco plants reported in these studies are greater than those previously shown to produce toxic effects in various plant species. In addition, tobacco plants are characterized by their large leaf area and rapid plant growth supported by a fibrous root system. Mature tobacco plants have leaf areas ranging from 0.09 to 0.14  $\text{m}^2$ , however in some cases a single cigar tobacco plant can produce as much as 2.3  $\text{m}^2$  of leaf area.<sup>12</sup> This allows large amounts of perchlorate to be stored in the various leaf tissues.

Understanding the potential for the uptake, translocation, and accumulation of toxic chemicals by plants is an important public health and ecological issue. Predictive tools are needed to understand and interpret the behavior of chemicals in the plants.<sup>13</sup> It is impractical to test the efficiency of all plant species under greenhouse conditions to phytoremediate chemical contaminants. Mathematical models, which combine the physiochemical properties of a chemical with the anatomical and physiological properties of plants, can be used as a predictive tool to assess the temporal aspects of phytoremediation and the efficiency of plants to phytoremediate environmental contaminants.

Plant uptake, translocation, and accumulation of environmental contaminants is very dependent on the anatomy and physiology of plants. The features that enable plants to

accumulate nutrients and water from soil also enable them to accumulate anthropogenic chemicals or substances.<sup>13</sup> With the exception of some hormone-like chemicals, plant uptake and translocation of anthropogenic chemicals appears to be carried out by passive transport (mass flow or diffusion). There is no evidence for active transport of anthropogenic chemicals as they move through the endodermis of the root and into the vascular system of plants.<sup>14</sup>

A few mathematical models have been developed to describe the fate of chemicals in plants. Boersma *et al.*,<sup>15,16</sup> and Trapp *et al.*<sup>13</sup> developed mathematical models to describe the uptake and translocation of organic chemicals. These authors validated the models with experiments based on uptake of bromacil by soybean plants. Model validations were performed assuming steady-state conditions with respect to plant growth and transpiration rates. A fugacity model was developed by Hung and Mackay<sup>17</sup> to predict the uptake and kinetics of organic chemicals in herbaceous agricultural plants. More recently, Zhang *et al.*<sup>18</sup> constructed a model to describe the transport of methyl *tert*-butyl ether (MTBE) into alfalfa plants. These authors used the model to estimate a diffusion coefficient for the transfer of MTBE from the root system into the stem. No models were found in the literature for the uptake of inorganic chemicals, including perchlorate, in plants. Unlike organic chemicals that have been previously modeled, perchlorate does not appear to degrade in plant tissues and is not expected to be lost to the atmosphere through transpiration. These factors introduce simplifying assumptions that reduce the number of model parameters and compartments compared to previously developed plant chemical fate models.

In this study, tobacco plants were grown in perchlorate amended hydroponics nutrient solutions where applied perchlorate concentrations were known and uptake levels could be monitored. Perchlorate concentrations in roots, stems, and leaves of tobacco plants were determined by ion chromatography (IC). A simple mathematical model was developed to describe the uptake, translocation, and accumulation of perchlorate in tobacco plants under rapid plant growth conditions. For the application of fate models for phytoremediation purposes, plant growth is an important process to include as the plants used for phytoremediation would likely be in their rapid growth phase.

## 2. Experimental procedures

Six flats of 250 *Nicotiana tabacum* var. K326 seedlings per flat were obtained from the Coastal Plains Experimental Station in Tifton, Georgia, in mid-April 2002. The six-week old seedlings were placed in a greenhouse at the EPA Ecosystem Research Division in Athens, Georgia. They were watered once or twice daily, depending on sun and heat exposure, with a hydroponics solution made by adding 47.85 g of Peter's 5-11-26 Hydrosol (Hummerts, St. Louis, MO, Cat. #07-5570), 31.65 g of calcium nitrate 15.5-0-0 (Hummerts, St. Louis, MO, Cat. #07-0355), and 50 L of water. All water used throughout the study was 18  $\text{M}\Omega$  cm water. When the seedlings reached 8–10 cm in height, or approximately 10 weeks of age, 200 seedlings of equal height and stem width were removed from the flats and the soil was gently and thoroughly rinsed from the roots with water. The seedlings were placed into white plastic 32 oz. Sweetheart deli/food containers (Thornton Bros., Athens, GA, Cat. #GW33TH) containing approximately 850 mL of the hydroponics solution. A 0.75 inch hole was drilled into each container snap-cap lid, through which the stems and roots were placed. Each container and lid was wrapped in aluminium foil to prevent light from penetrating through the white plastic. Small pieces of foil were wrapped around the base of the stems to help provide support.

The growth containers were refilled with the hydroponics

solution on a daily basis for four weeks while the tobacco plants acclimated to the solution. The hydroponics solutions used for the uptake studies were prepared as previously described but in addition, perchlorate was added to give the desired concentrations at the start of the studies.

### 2.1 13-Day plant uptake study

After 4 weeks of growing in the hydroponics solution, 49 plants of equal height and stem width were chosen for the phytoremediation study. Twenty-one plants were placed into 800 mL of 25 ppm and 75 ppm perchlorate solutions. All perchlorate-amended nutrient solutions were made up in single batch lots and perchlorate concentrations were verified using ion chromatography prior to dispensing the solution into the plastic containers. Seven control plants remained in perchlorate-free hydroponics solution.

Seven harvest days were designated at 1, 3, 5, 7, 9, 11, and 13 days after treatment with perchlorate. Three plants per concentration and one control were harvested each harvest day, as described later in this section. During this 13-day period, measured volumes of perchlorate-free nutrient solution were added to each container to replace the volume of nutrient solution taken up by the plant.

### 2.2 Plant uptake of low concentrations (ppb) of perchlorate

In a separate study, the ability of the tobacco plant to phytoremediate perchlorate at very low concentrations (parts-per-billion) was studied. Six plants of equal height and stem width were transferred to containers with 800 mL of water fortified with 10 ppb perchlorate and 100 ppb perchlorate (three plants each). The ionic matrix of the nutrient solution required a 1/1000 dilution before IC analysis. Therefore, unlike studies 2.1, 2.3, and 2.6, these plants were grown in water without the nutrient solution, which allowed the detection of low concentrations of perchlorate in the water at the end of the growth study. Two plants grown in water without treatment served as controls, and the tobacco plants were replenished with measured volumes of perchlorate-free water when needed. At the end of the 14-day growth period, the plant roots were rinsed into the plastic containers and the solutions were brought up to the initial 800 mL volume. Aliquots of the solutions were analyzed to determine the amounts of perchlorate left in the solutions.

### 2.3 Plant uptake of high concentrations (ppm) of perchlorate

In the preliminary study at this laboratory, tobacco plants showed signs of toxicity with 100 ppm perchlorate within 12 days of exposure (data not shown). To verify this observation, six plants of equal height and stem width were transferred to containers containing 850 mL of solution with 100 ppm perchlorate derived from sodium perchlorate and ammonium perchlorate (three plants each). Two plants were maintained as controls. The plants were grown for 14 days, and the nutrient solutions were replenished as needed with measured volumes of perchlorate-free solution. At the end of the 14-day growth period, the solutions were brought up to the initial 850 mL volume. Aliquots of the solutions were analyzed to determine the amounts of perchlorate left in the solutions. These plants were not harvested, however visual signs of toxicity were noted.

### 2.4 Plant extraction methods

At 12:00 noon on the designated harvest days, the tobacco plants were brought into the lab for harvesting. The leaves were removed from the stem at the base of the petioles using a razor blade. Each leaf was placed on a clean glass plate and individually cut down the center of the midrib, then sliced into half-inch square pieces. The cut leaf material, including the

midrib, was placed into a pre-weighed plastic bag, and the total leaf fresh weight was obtained using a Mettler AE240 balance. The stem and roots of the plant were removed from the container and the roots were placed over a 1 L graduated cylinder to drain. The remaining hydroponics solution was also poured into the 1 L cylinder and the volume was recorded to determine the amount of solution that was transpired the previous day. The root mass was rinsed with water into the graduated cylinder until the volume was brought up to the original starting volume of 800 mL for Studies 2.1 and 2.2, and 850 mL for Studies 2.3 and 2.6. Samples of the hydroponics solutions were taken and stored at 4 °C until analysis. As a precautionary measure, the roots were further rinsed with four 1 L portions of water each to ensure removal of any residual perchlorate. These rinses were discarded since they contained only 1–5 ppb perchlorate. The stem and roots were dried using paper towels, and the roots were sectioned off from the stem at the start of the first lateral root. The stem was cut with a razor blade into 2 mm cross sections, placed into a pre-weighed plastic bag, and the total stem fresh weight was obtained. The roots were cut into 1 cm pieces using scissors, placed into a pre-weighed glass bottle, and the total root fresh weight was obtained. All plant material was freeze-dried for 48–72 h and the weight recorded. This harvesting procedure was repeated for each plant, starting with the controls and ending with the highest perchlorate concentration.

A method for the analysis of perchlorate in plants was developed, based on dry and fresh weight, by Ellington and Evans.<sup>19</sup> In this method, freeze-dried and ground samples were extracted with water and heated to precipitate proteins. The aqueous phase was centrifuged, exposed to alumina, and filtered through three cartridges filled with divinylbenzene to yield a water-clear extract for IC analysis. This method was chosen because it allows the quantitative analysis of perchlorate with little to no plant matrix interference.

### 2.5 Model structure and development

The PK model defined the plant as a set of compartments based on the anatomy and physiology of the plant. The tobacco plant was divided into five compartments: solution, roots, stem, stem storage, and leaves (Fig. 1). The compartments were described by mass balance differential equations and plant-specific physiological parameters.

The PK model was developed using Advanced Continuous Simulation Language (ACSL) Version 11.8.4 (AEGIS Technologies, Huntsville, AL). To mathematically describe the

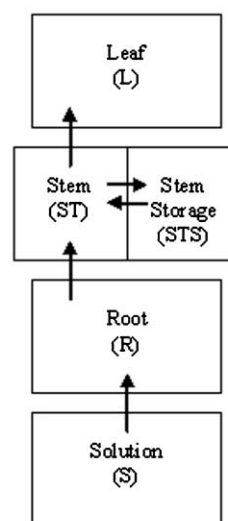


Fig. 1 Sequence of plant compartments based on plant anatomy and physiology used in the PK model.

**Table 1** PK Model equations

| Compartment  | Mass balance equation   |
|--------------|---|
| Solution     | $dA_S/dt = -R_S(t) \times C_S$ where $C_S = A_S/V_S$  |
| Root         | $dA_R/dt = [R_S(t) \times C_S] - [DF_R(t) \times C_R]$ where<br>$DF_R(t) = DF \times R_S(t)$ and $C_R = A_R/M_R(t)$                                   |
| Stem         | $dA_{ST}/dt = [DF_R(t) \times C_R] - [T_{ST} - A_{ST}] +$<br>$[T_{STS} \times A_{STS}] - [R_S(t) \times C_{ST}]$ where<br>$C_{ST} = A_{ST}/M_{ST}(t)$ |
| Stem storage | $dA_{STS}/dt = [T_{ST} \times A_{ST}] - [T_{STS} \times A_{STS}]$   |
| Leaf         | $dA_L/dt = [R_S(t) \times C_S]$   |

processes within a living plant using mass balance differential equations, a few simplifying assumptions were made: (a) movement of perchlorate between compartments occurs by mass flow with the transpiration stream or by diffusion, (b) phloem transport of perchlorate from the leaves back down to the roots is negligible, (c) no significant degradation of perchlorate occurs in the plant system, and (d) no evaporative loss of perchlorate occurs in the stomatal activity of the leaves.

The PK model is comprised of a set of mass balance equations, one for each plant compartment (Table 1). Model terms and definitions used in the model can be found in Table 2. The rate of uptake of the hydroponics solution into the plant was estimated by measuring the rate of solution utilization ( $R_S(t)$ , L per day) in the 13-day plant uptake study. The rate of uptake of perchlorate into the root system from the solution and the accumulation of perchlorate in the leaf tissue were described as flow-limited processes, governed by the transpiration stream. Clearance of perchlorate from the root tissue was described as a diffusion-limited process, where diffusion across the cell membranes of the endodermis was the rate-limiting step. In the stem, perchlorate distribution from the xylem into the stem storage tissue and *vice versa* was described as a bi-directional first-order diffusional process.

The PK model assumed a constant supply of nutrient solution. The xylem flow rate was assumed to equal measured average daily nutrient solution utilization rates. The xylem flow rate and plant growth curves were determined by linear interpolation of measured solution utilization rates and plant weights at two-day intervals during the 13-day plant uptake study (refer to Supplementary Data Figs S1 and S2†). Linear

**Table 2** Symbols and subscripts used in the PK model mass equations

| Symbol    | Definition  |
|-----------|---|
| $R(t)$    | Xylem flow rate/L per day                               |
| $T$       | Transfer parameter per day                              |
| DF        | Diffusion factor (unitless)                             |
| $DF(t)$   | Diffusion factor, dependent on time/L per day           |
| $V$       | Volume/L  |
| $M(t)$    | Mass (wet weight), dependent on time (kg)               |
| $C$       | Concentration of $ClO_4^-$ /mg $L^{-1}$ or mg $kg^{-1}$ |
| $A$       | Amount of $ClO_4^-$ /mg                                 |
| Subscript | Definition  |
| S         | Solution  |
| R         | Roots   |
| ST        | Stem  |
| STS       | Stem Storage  |
| L         | Leaf  |

**Table 3** Model calibration and plant anatomy parameters

| Parameter | Value              | Description                                      | Source                              |
|-----------|--------------------|--|-------------------------------------|
| $T_{ST}$  | $16.33 \pm 2.74^a$ | Transfer parameter from ST to STS                | Optimized <sup>b</sup>              |
| $T_{STS}$ | $0.31 \pm 0.06^a$  | Transfer parameter from STS to ST                | Optimized <sup>b</sup>              |
| DF        | $0.40 \pm 0.02^a$  | Diffusion factor                                 | Optimized <sup>b</sup>              |
| $P_{ST}$  | 0.07               | Proportion of total stem weight allocated to ST  | Boersma <i>et al.</i> <sup>15</sup> |
| $P_{STS}$ | 0.93               | Proportion of total stem weight allocated to STS | Boersma <i>et al.</i> <sup>15</sup> |

<sup>a</sup>Values of  $T_{ST}$ ,  $T_{STS}$ , and DF  $\pm$  standard deviation. <sup>b</sup>Parameters were fit to calibration data by maximum likelihood estimation.

interpolations were implemented in ACSL using a TABLE function.

The root diffusion factor was optimized by maximum likelihood estimation as implemented in ACSL Math Version 11.8.4 (AEgis Technology, Huntsville, AL). The Nelder-Mead algorithm was used for likelihood estimation. The error model was fit to the experimental data. The starting value for the DF parameter was found by visually fitting the model to the data prior to running the optimization. The two perchlorate concentrations (25 ppm and 75 ppm) were first fit separately and then simultaneously to the data for parameter estimation (Table 3). There was successful convergence to final parameter estimates.

The values of the transfer parameters (unitless) between the stem and stem storage compartments were optimized by maximum likelihood estimation as described above (Table 3). Proportions of measured total stem weights from experimental data allocated to the stem and stem storage were 0.07 and 0.93, respectively, as determined for a normalized dicot plant by Boersma *et al.*<sup>16</sup>

## 2.6 Model validation study

To validate the PK model, a fourth uptake study was completed. Procedures of this study followed those of the 13-day plant uptake study (Section 2.1), with the exception that the plants in this study were grown in 850 mL of 10, 50, 75, and 100 ppm perchlorate amended nutrient solution for a two-day period. Three plants per concentration and two controls were harvested two days after treatment began.

## 3. Results and discussion

### 3.1 13-Day plant uptake study

The uptake, translocation, and accumulation of perchlorate in tobacco plants were determined in this study. Volumes of nutrient solution taken up by individual plants were recorded daily during the 13-day plant uptake study. After 1 day of treatment, the plants (controls, 25 ppm, and 75 ppm perchlorate amended plants) had taken up an average of  $148 \pm 31$  mL and after 13 days of treatment,  $3714 \pm 310$  mL were translocated throughout the plant. In general, the total plant fresh weights doubled from an average of  $90.8 \pm 8.4$  g after 1 day to  $208.6 \pm 11.1$  g after 13 days. Total plant dry weights increased from an average of  $11.0 \pm 1.9$  g after 1 day to  $26.4 \pm 1.1$  g after 13 days, a 2.5 fold increase. The study was terminated after 13 days of treatment when the plastic containers could no longer support the rapidly growing tobacco plants.

Plant variability in size and health was observed throughout the growth studies. Due to this plant variation, the averages of two of the three plants in each concentration and harvest group were used in the final results. Determination of which two plants were to be used was based on solution uptake by the roots, fresh and dry weights, and the overall appearance and health of the plants. Plant variability may be due to physiological variations or to the lack of aeration of the nutrient solution over the 13 days.

The results of this phytoremediation study showed that

perchlorate was taken up by the root system, traveled up the stem *via* the xylem, and probably accumulated in the vacuoles of the various cell types in the leaves and stems. The highest perchlorate content found in the roots was  $1.1 \pm 0.02$  mg after 1 day, but then decreased as perchlorate was taken up into the stems and leaves (refer to Supplementary Data Table S1†). The perchlorate content in the stem remained fairly constant, ranging from  $0.5 \pm 0.1$  mg to  $0.8 \pm 0.1$  mg in the 25 ppm perchlorate amended plants, and from  $1.2 \pm 0.1$  mg to  $1.9 \pm 0.3$  mg in the 75 ppm perchlorate amended plants. Perchlorate accumulated in the leaves over the 13-day growth period to  $18.3 \pm 1.5$  mg and  $54.4 \pm 2.8$  mg in the 25 ppm and 75 ppm perchlorate amended plants, respectively. The perchlorate content in the nutrient solution decreased over time from  $16.7 \pm 0.4$  mg to  $0.4 \pm 0.5$  mg in the 25 ppm perchlorate amended plants and from  $47.8 \pm 1.8$  mg to  $2.5 \pm 1.4$  mg in the 75 ppm perchlorate amended plants. Fig. 2 clearly shows these relationships for the 25 ppm and 75 ppm perchlorate amended plants.

After the 13-day phytoremediation study in the perchlorate amended nutrient solution, 95.4% of the total perchlorate applied was taken up by the 25 ppm perchlorate amended plants, and 92.7% of the total perchlorate applied was in the 75 ppm perchlorate amended plants. Most of the remaining perchlorate was accounted for in the amended nutrient solution. The highest perchlorate concentration found in the plants was  $3700.9 \pm 13.9$  mg kg<sup>-1</sup> dry weight on day 11 in the leaf, where 0.37% of the dry matter was perchlorate (refer to Supplementary Data Table S2†). This suggests that tobacco plants may be effective for the phytoremediation of perchlorate from the environment. After 9 days, very little additional perchlorate was accumulated in the leaves which can be attributed to the near-depletion of perchlorate in the nutrient solution. However, the plants continued to grow which added

to the total mass of the plant. This resulted in the dilution of perchlorate on a dry or fresh weight basis within the plants. Perchlorate recoveries ranged from 97.4% to 107.6% in the 25 ppm perchlorate amended plants and nutrient solutions, and from 92.0% to 99.8% in the 75 ppm plants and nutrient solutions. These high recoveries suggest that the perchlorate is being stored in the plant and that the degradation of perchlorate is negligible, if degradation occurs. The lack of degradation can be attributed to the energy required to degrade the perchlorate anion and/or the lack of the proper degradation enzyme(s) in plant tissues. Assuming there is no degradation of perchlorate, the recovery errors may be the result of methodology.

### 3.2 Plant uptake of low concentrations (ppb) of perchlorate

Tobacco plants were shown to be very effective in phyto-remediating parts-per-billion concentrations of perchlorate, which are commonly found in the environment. In Table 4, two of the three 10 ppb perchlorate amended plants' water concentrations were below the method detection limit of the IC (0.5 ppb), indicating that at least 95% of the perchlorate was taken up by the plants. The third plant exposed to 10 ppb had to be discarded due to disease. Two of the three 100 ppb perchlorate amended plants' water concentrations were below the detection limit as well. The third plant's water had 0.0021 mg perchlorate (2.7% of the total perchlorate applied) remaining after the 14 days, indicating that 97.3% of the total perchlorate applied was taken up by the plant. Chlorosis was evident in these water-grown plants after approximately 6 days due to the lack of nutrients during the 14 day plant uptake study. However, this did not affect perchlorate uptake by the plants.

### 3.3 Plant uptake of high concentrations (ppm) of perchlorate

The sodium and ammonium salts of perchlorate were used to determine whether any toxicity was due to the perchlorate or excess sodium ions. Table 4 shows that three out of the four plants exposed to 100 ppm perchlorate had less than 2% of the total perchlorate applied remaining after the 14 days. The fourth plant had approximately 10% of the perchlorate remaining in the solution, most likely due to physiological variations of the plant. No signs of toxicity were observed during the study for either sodium or ammonium perchlorate amended plants. Although the preliminary study had suggested that a concentration of 100 ppm perchlorate might be toxic to the plants, the age at which the plants were exposed must be considered. The plants in the preliminary study were exposed at 10 weeks of age, as opposed to this study's plants being exposed at 14 weeks of age. This implies that the toxicity of perchlorate may be related to the stage of maturity and size of the tobacco plants at the time of exposure to perchlorate.

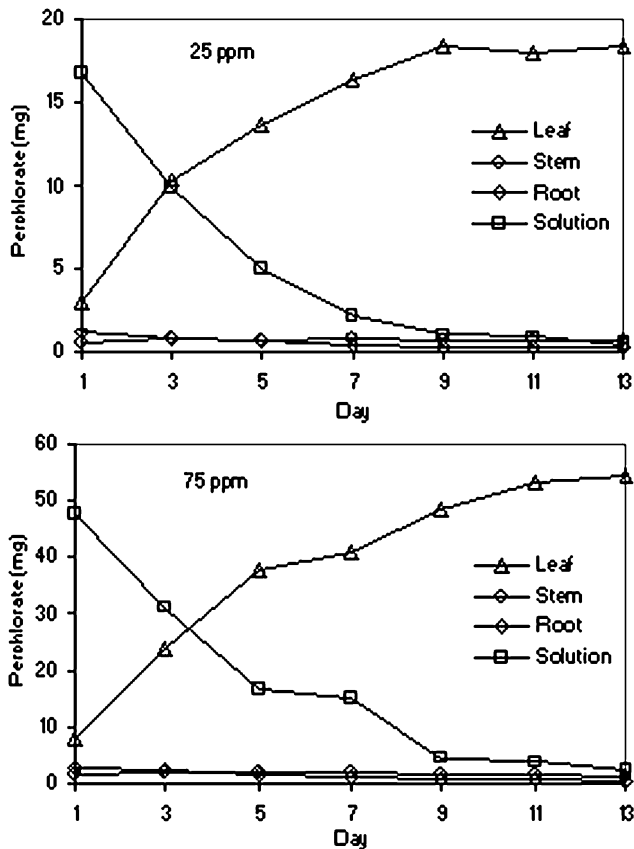


Fig. 2 The distribution of perchlorate in 25 ppm (top) and 75 ppm (bottom) perchlorate amended tobacco plants and depletion of perchlorate from the nutrient solutions ( $n = 2$ ).

Table 4 Perchlorate remaining in solution after 14 days

| Plant   | ClO <sub>4</sub> <sup>-</sup> (mg) | ClO <sub>4</sub> <sup>-</sup> (ppm) |
|---|------------------------------------|-------------------------------------|
| 10 ppb <sup>a</sup> Plant 1                                   | ND <sup>b</sup>                    | ND                                  |
| 10 ppb Plant 2  | ND                                 | ND                                  |
| 100 ppb <sup>c</sup> Plant 1                                  | ND                                 | ND                                  |
| 100 ppb Plant 2   | 0.002                              | 0.0027                              |
| 100 ppb Plant 3   | ND                                 | ND                                  |
| 100 ppm <sup>d</sup> NaClO <sub>4</sub> Plant 1               | $1.6 \pm < 0.1^e$                  | $1.9 \pm < 0.1$                     |
| 100 ppm NaClO <sub>4</sub> Plant 2                            | $8.9 \pm 0.2$                      | $10.4 \pm 0.3$                      |
| 100 ppm <sup>f</sup> NH <sub>4</sub> ClO <sub>4</sub> Plant 1 | $1.1 \pm < 0.1$                    | $1.2 \pm < 0.1$                     |
| 100 ppm NH <sub>4</sub> ClO <sub>4</sub> Plant 2              | $2.0 \pm < 0.1$                    | $2.3 \pm < 0.1$                     |

<sup>a</sup>Actual initial ClO<sub>4</sub><sup>-</sup> concentration was 9.7 ppb (7.8 μg). <sup>b</sup>Non detectable. <sup>c</sup>Actual initial ClO<sub>4</sub><sup>-</sup> concentration was 97.7 ppb (78.2 μg). <sup>d</sup>Actual initial ClO<sub>4</sub><sup>-</sup> concentration was 102.2 ppm (86.9 mg). <sup>e</sup>Milligrams ClO<sub>4</sub><sup>-</sup> ± standard deviation. <sup>f</sup>Actual initial ClO<sub>4</sub><sup>-</sup> concentration was 101.4 ppm (86.2 mg).

There are a few limitations to the use of tobacco for phytoremediation. Tobacco plants would be limited to phytoremediating perchlorate contaminated water within the rhizosphere, from sources such as irrigation or surface water, where growth conditions are appropriate for tobacco plants. Since tobacco roots don't extend beyond the rhizosphere, these plants would be ineffective in phytoremediating perchlorate contaminated groundwater. Also, since the accumulated perchlorate is not degraded in the tobacco plant, the plants would have to be removed after remediation of the perchlorate. Incineration may be an effective method to dispose of perchlorate contaminated plants since perchlorate decomposes at around 300 °C.<sup>3</sup>

### 3.4 Plant kinetic model

The predicted perchlorate uptake from the hydroponics solution amended with 25 and 75 ppm perchlorate closely followed the measured values from the 13-day plant uptake study (Fig. 3). Perchlorate concentrations in the plant roots (Fig. 4) increased rapidly during the first day of exposure. This can be attributed to the initial entry of perchlorate and nutrient solution into the root cortex, comprised of cells and free space in which water and solutes freely move. The filling of the cortex cells and free space occurs as soon as exposure is initiated due to the plant roots being completely bathed in the nutrient solution. Such behavior is not expected in soil-grown plants.<sup>20</sup> On the first modeling attempt, the amount of perchlorate in the roots was under-predicted compared to the measured values from the 13-day plant uptake study (Fig. 4). A parameter was added to the root compartment mass balance equation to adjust the perchlorate content in the root tissue entering the root xylem and stem xylem (Table 3). To mathematically describe the diffusion of perchlorate from the root tissue,  $R_S(t)$

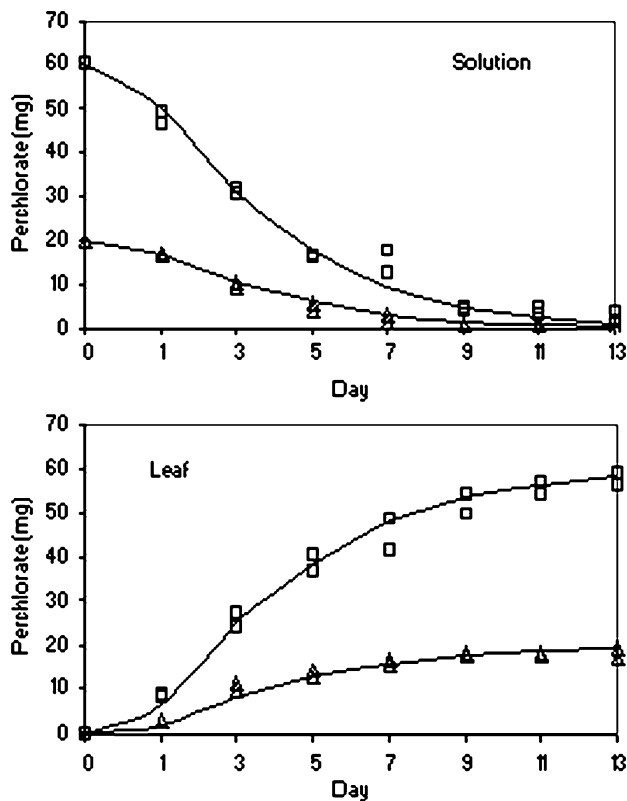


Fig. 3 Perchlorate content in the solution (top) and leaves (bottom) exposed to 25 ppm and 75 ppm perchlorate. Smooth lines follow model simulations (time-step of 0.25, or four times per hour), triangles indicate measured values from the 13-day plant uptake study at 25 ppm, and squares indicate measured values from the 13-day plant uptake study at 75 ppm.

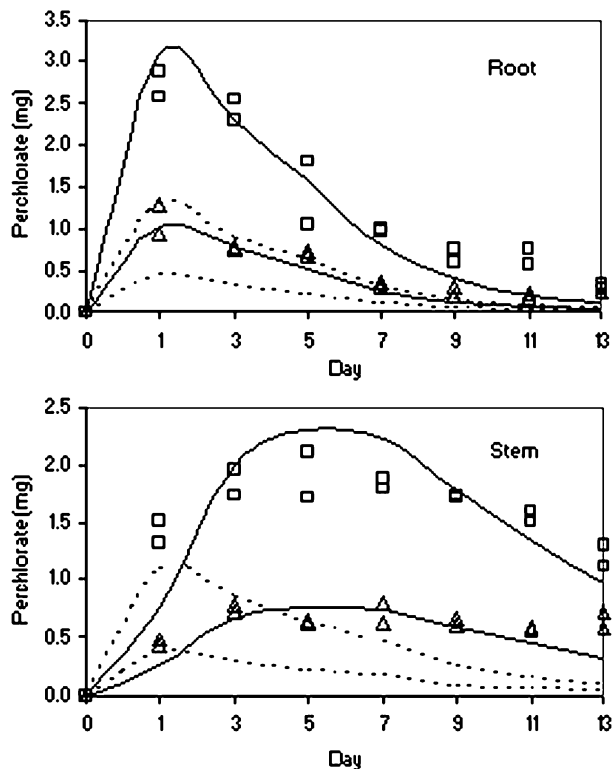


Fig. 4 Perchlorate content in the roots (top) and stems (bottom) exposed to 25 ppm and 75 ppm perchlorate. Smooth lines follow model simulations (time-step of 0.25, or four times per hour), dotted lines follow model simulations without added parameters, triangles indicate measured values from the 13-day plant uptake study at 25 ppm, and squares indicate measured values from the 13-day plant uptake study at 75 ppm.

(L per day) was multiplied by a diffusion factor (DF, unitless). Thus, the diffusion of perchlorate out of the root tissue was assumed to be proportional to the increase in the transpiration rate, reflecting growth of the plant. This approach is mathematically equivalent to the approach taken previously by Trapp *et al.*,<sup>13</sup> where DF is equivalent to the TSCF (Transpiration Stream Concentration Factor).

Perchlorate kinetic behavior in the stem (Fig. 4) is characterized by an initial increase in concentration during the first three days of exposure and retention in the stem. In the 75 ppm perchlorate amended plants, there was a gradual decrease in perchlorate concentration after day 9 as the perchlorate is depleted from the solution and accumulated in the leaves. By day 3, it appears that the stem perchlorate concentration in the 25 ppm perchlorate amended plants reached equilibrium with the concentration in the transpiration stream. On the first modeling attempt, the amount of perchlorate in the stem was under-predicted compared to the measured values from the growth study. Consequently, a stem storage compartment was added to the model to better describe perchlorate retention in the stem. Perchlorate transfer between the stem xylem and stem storage was described as an unsymmetrical bi-directional first-order process to represent diffusion of perchlorate in and out of the stem storage compartment. These first-order constants allowed for the description of a short retention of perchlorate until the concentrations of the stem xylem and stem storage reached equilibrium, at which time perchlorate was released from storage and further translocated up the stem xylem into the leaves. Peak stem concentration was dependent on perchlorate concentration, and no saturation point was observed.

There are a few possible explanations for the retention of perchlorate in the stem storage compartment. This compartment is comprised of cortex and pith tissues of the stem, both of

which contain plant cells. It is possible that perchlorate in the cortex and pith tissue diffuses through the cell membranes and enters the cell. Within the cell, perchlorate can be drawn into the vacuoles, which, like the vacuoles in the leaf mesophyll cells, are capable of accumulating water and dissolved inorganic ions. The retention could be due to ionic attraction or interaction of perchlorate with cations within the vacuoles. In addition, a very small fraction of perchlorate may bind to cell proteins.

Perchlorate accumulation in the leaves (Fig. 3) was modeled without the addition of calibration parameters. Thirteen days after exposure, the model predicts that 96.3% of both the 20.13 mg perchlorate applied in the 25 ppm perchlorate amended plants and of the 60.34 mg perchlorate applied in the 75 ppm perchlorate amended plants will be accumulated in the leaves. In the 13-day phytoremediation study, 90.8% of the 25 ppm and 95.2% of the 75 ppm perchlorate amended plants was accumulated in the leaves 13 days after exposure.

There are a couple of limitations of the PK model for the uptake, translocation, and accumulation of perchlorate by tobacco plants. Instantaneous exposure to perchlorate, as observed in a hydroponics growth study, would not occur under typical field conditions except where a spill of contaminated water would reach the root zone of the plants in a very short period of time. For this type of condition, the model should be run as presented here. However, for field conditions in which there is a low level, chronic exposure to perchlorate, such as plants growing in contaminated soils, the model parameters should be adjusted accordingly. A slower rate of uptake would be expected as roots interface with soil solution, vapor, and solid phases rather than a hydroponics solution. In addition, for the purpose of simplifying the model, the compartments were assumed to be homogeneously mixed compartments. In field conditions, there may be significant differences between upper and lower sections of the plant roots, stems, and leaves, within the xylem as well as in storage tissues. Greater variability in the uptake of perchlorate in field conditions would be expected due to varying soil properties, such as pH, organic matter, and moisture content.

### 3.5 Model validation

The 2-day plant uptake study was used to validate the PK model. Measured and predicted amounts of perchlorate are given in Table 5. Across the four concentrations, 10, 50, 75, and 100 ppm perchlorate, all but two of the predicted values were within the calculated 95% confidence intervals of the measured values. In the 75 ppm perchlorate amended plants, perchlorate content in the leaf was under-predicted. In the 100 ppm perchlorate amended plants, perchlorate content in the root was over-predicted. These results suggest that the PK model is most reliable for perchlorate concentrations less than 75 ppm. The pharmacokinetics of perchlorate in the stem appears to be complex and may be governed by a saturable transport process. Further research is needed to gain a better understanding of perchlorate kinetics in the tobacco plant at high perchlorate concentrations.

The PK model can be applied to other dicot vascular plants that are tolerant of perchlorate by measuring plant wet weights and nutrient solution utilization rates throughout the period of perchlorate accumulation. The PK model can also be applied to similar nonvolatile, nondegradable inorganic chemicals for prediction of accumulation in plants. For predicting root and stem translocation, calibration parameters should be estimated from plant tissue kinetic data. The PK model could then be used as a screening tool for phytoremediation or environmental risk applications. More specifically, the PK model can aid in field trial design for testing tobacco's ability to phytoremediate perchlorate.

In conclusion, *Nicotiana tabacum* appears to be a potential

**Table 5** Predicted perchlorate content from the PK model and measured perchlorate content after 2 days of treatment in validation study

|                      |          | Perchlorate (mg) |                       |
|----------------------|----------|------------------|-----------------------|
|                      |          | Predicted        | Measured <sup>a</sup> |
| 10 ppm <sup>b</sup>  | Leaf     | 2.24             | 2.71 (1.99–3.43)      |
|                      | Stem     | 0.21             | 0.19 (0.07–0.31)      |
|                      | Root     | 0.39             | 0.37 (0.20–0.54)      |
|                      | Solution | 5.97             | 5.88 (5.26–6.50)      |
| 50 ppm <sup>c</sup>  | Leaf     | 10.85            | 13.27 (6.79–19.75)    |
|                      | Stem     | 1.03             | 0.92 (0.94–2.78)      |
|                      | Root     | 1.89             | 1.72 (1.43–2.01)      |
|                      | Solution | 28.91            | 29.31 (23.86–34.76)   |
| 75 ppm <sup>d</sup>  | Leaf     | 16.27            | 21.32 (16.28–26.36)   |
|                      | Stem     | 1.55             | 1.48 (1.19–1.77)      |
|                      | Root     | 2.83             | 2.18 (1.13–3.23)      |
|                      | Solution | 43.37            | 42.72 (35.82–49.62)   |
| 100 ppm <sup>e</sup> | Leaf     | 21.61            | 27.88 (21.60–34.16)   |
|                      | Stem     | 2.05             | 1.92 (1.51–2.33)      |
|                      | Root     | 3.76             | 2.76 (1.85–3.67)      |
|                      | Solution | 57.59            | 56.66 (52.36–60.96)   |

<sup>a</sup>Measured values from validation study and 95% confidence intervals,  $n = 3$ . <sup>b</sup>Actual initial  $\text{ClO}_4^-$  concentration was 10.36 ppm (8.81 mg). <sup>c</sup>Actual initial  $\text{ClO}_4^-$  concentration was 50.21 ppm (42.68 mg). <sup>d</sup>Actual initial  $\text{ClO}_4^-$  concentration was 75.32 ppm (64.02 mg). <sup>e</sup>Actual initial  $\text{ClO}_4^-$  concentration was 100.02 ppm (85.02 mg).

candidate for the phytoremediation of perchlorate. The tobacco roots are fibrous with large surface areas for the uptake of perchlorate, the observed storage capacity (0.37%) of perchlorate in the leaves is substantial, and the plants are fairly tolerant of initial perchlorate concentrations up to 100 ppm. Tobacco plants were shown to effectively accumulate perchlorate over a wide range of initial concentrations (10 ppb to 100 ppm) from the hydroponics solution. A simple five compartment mathematical model was developed to describe the distribution of perchlorate in tobacco plants under non-steady state plant growth conditions. The PK model adequately predicts perchlorate uptake by roots from the nutrient solution, translocation into the xylem by diffusion across the root endodermis cell membranes, transfer between the stem xylem and stem storage, and accumulation in plant leaves.

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