

5.3 PLANTS

5.3.1 Laboratory Studies

5.3.1.1 Introduction

Evaluation of the potential movement of contaminants from water and/or soil to vegetation is critical for determining exposure routes to wildlife and humans. The conditions under which contaminants move from soil/sediment to plants are often initially characterized in the laboratory or greenhouse where the environment is more easily controlled and plant species can be selected for comparative purposes. Perchlorate is very water soluble and likely to move readily to vegetation. The uptake and distribution of perchlorate in plants was evaluated in short-term laboratory experiments. These tests provided the basis for determining uptake factors for perchlorate in the environment.

5.3.1.2 Methodology

5.3.1.2.1 Reagents and Standards

A 100 µg/mL certified sodium perchlorate solution (Accustandard, Inc) was purchased to prepare perchlorate calibration standards. Sodium hydroxide was purchased from Fisher Scientific and diluted to make the eluent required for the ion chromatography. Hydrosol is a diluted solution of Peters All Purpose Plant Food.

5.3.1.2.2 Uptake Experiments

Cucumber, lettuce, and soybean were selected for the uptake experiments. Seeds of plants were placed in polystyrene cups containing 50 g or 100 g Ottawa sand (Fisher Scientific) and grown in an incubator at 22 °C (15:9 light:dark photoperiod). The cups were covered with Petri dishes to avoid excessive loss of water. Our initial studies indicated that plant germination was not affected by up to 1000 ppb perchlorate (ng perchlorate/g sand). Since 100 ppb is a more environmentally relevant concentration, all uptake experiments were conducted at this concentration. The experimental design varied according to different plant species. For cucumber, three four-week experiments were conducted in the presence of varying ratios of Hydrosol: (1) 100% Hydrosol, (2) Hydrosol:Milli-Q water (50:50) and (3) Hydrosol: Milli-Q water (25:75). Due to our concern about the lack of nutrition for proper plant growth, another experiment in which cucumber was watered with 100% Milli-Q water lasted only two weeks. An eight-week cucumber uptake study was also conducted in which the sand was respiked with perchlorate after week 4. Lettuce was grown in the presence of 100% Hydrosol for six weeks. For soybean, two four-week experiments were conducted: (1) 100% Hydrosol, and (2) Hydrosol: Milli-Q water (50:50). There were four plants in each treatment group at every sampling point. Four control plants of the appropriate species (no perchlorate) were included for each of the respective uptake experiments. An additional control with perchlorate and no plant was sampled each week to assess possible microbial transformation of perchlorate in the sand.

Plants were removed each week and sectioned into two parts: portion of plant above sand level (primarily leaves) and portion of plant below sand level (primarily roots). Each portion of the plant sample was weighed, rinsed with water, and allowed to dry prior to extraction (described below). The amount of perchlorate remaining in sand was also determined weekly by adding a known volume of Milli-Q water (18MΩ) to the cups, and extracting the contents by mechanical agitation. Sand extracts were analyzed by ion chromatography (described below).

5.3.1.2.3 Tissue Extraction and Extract Cleanup

All plants were extracted in 11-mL cells using Milli-Q water (18MΩ) with a Dionex (Sunnyvale, CA) Accelerated Solvent Extractor (ASE 200) using the following procedure (Anderson and Wu, 2002). Cells were heated for 5 min at 100° C, filled with Milli-Q water, and pressurized to 1500 psi. Total extraction time was 15 min. At the completion of the extraction procedure, extract volume was recorded. For all plants, 1.0 mL of extract volume was cleaned with alumina solid phase extraction (SPE) cartridges and then diluted to 5 mL with Milli-Q water. Finally, the diluted extracts were filtered with a 0.45 μm Acrodisc® into 5-mL ion chromatography vials.

5.3.1.2.4 Analysis

All samples (plasma extracts, tissue extracts, and surface water) were analyzed by ion chromatography similar to EPA Method 314 (See **Appendix X** for specific procedures).

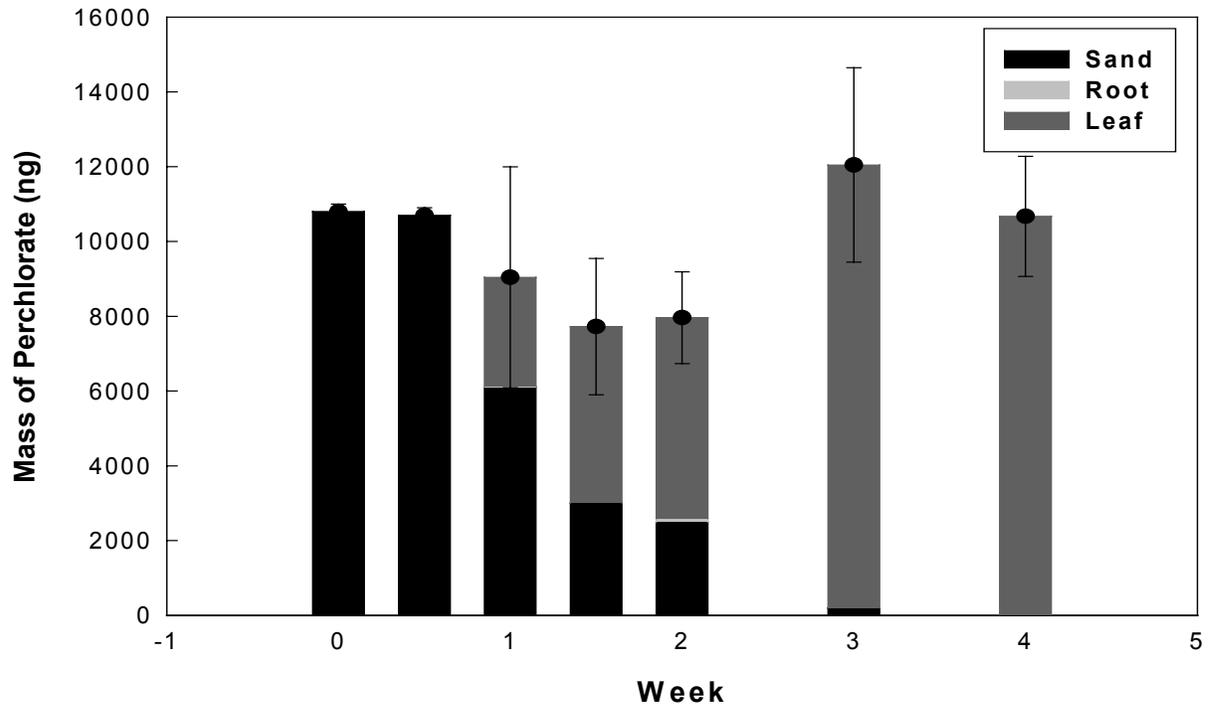
5.3.1.2.5 Data Analysis

All statistical tests were conducted using SAS software (Version 8). Comparisons of mean perchlorate concentrations between planted sand and unplanted sand were performed using Student's t-test. Two-way analysis of variance (ANOVA) was conducted to evaluate the effect of species on perchlorate uptake; Duncan's multiple range test was used after the ANOVA.

5.3.1.3 Data

Analysis of control samples (planted and unplanted cups without perchlorate) at the end of each experiment indicated that perchlorate was not detected in planted sand, roots, or leaves. In addition, loss of perchlorate from sand in the unplanted controls was negligible. For the uptake experiments, perchlorate was removed from sand by plants (**Figure 5-122**) and taken up in aboveground vegetation (**Table 5-20**) in all of the tests conducted.

In the four-week cucumber uptake experiment in the presence of Hydrosol, perchlorate concentration in the planted sand decreased to ND at Week 3. There was an 11% decrease in the unplanted sand during the same four weeks. Considerable uptake of perchlorate into cucumber leaves (**Table 5-20**) was observed. Similar results were obtained for the lettuce and soybean perchlorate uptake experiments. Comparisons of planted sand and unplanted sand in each of these experiments showed significant differences in perchlorate concentrations (P ranged from <0.0001 to 0.0401). There was only a slight decrease of perchlorate in unplanted sand over time, suggesting that microbial transformation and chemisorption/physisorption/ion exchange of perchlorate in the experimental system was negligible.



Error bars represent one standard deviation of total perchlorate

Figure 5-122
Perchlorate Distributions among Soil, Root and Leaves from a Four-week
Cucumber (*Cucumis sativus* L.) Perchlorate Uptake Experiment in the Presence of
Hydrosol:Milli-Q Water (25:75)

Table 5-20
Distribution of Perchlorate in the Test Systems

Plants	Sample Time	Perchlorate Concentration (ppb) ± SD				Mass of Perchlorate in Plant System (ng) ± SD
		Unplanted Sand	Planted Sand	Leaves	Root	
Cucumber						
Hydrosol(H)*	Week 0	111 ± 5 ^c	114 ± 2 ^c	NA	NA	11200 ± 250 ^c
	Week 1	NA	115 ± 14 ^b	35000 ± 9050 ^b	261200 ± 38900 ^b	13100 ± 1640 ^b
	Week 2	108 ± 0.68 ^b	9 ^a	20670 ^a	150800 ^a	2740 ^a
	Week 3	NA	ND ^c	22080 ± 17140 ^c	ND ^c	1200 ± 900 ^c
	Week 4	99 ^a	ND ^c	41060 ± 32010 ^c	ND ^c	2260 ± 1660 ^c
H*:Milli-Q (50:50)	Week 0	104 ± 2 ^c	104 ± 2 ^c	NA	NA	10400 ± 200 ^c
	Week 0.5	102 ± 10 ^c	102 ± 10	NA	NA	10200 ± 940 ^c
	Week 1	94 ^a	54 ± 19 ^d	98000 ± 44690 ^d	313900 ± 51400 ^d	8330 ± 3550 ^d
	Week 1.5	100 ^a	43 ± 17 ^d	159900 ± 33530 ^d	ND ^d	8350 ± 1780 ^d
	Week 2	106 ^a	26 ± 8 ^d	267700 ± 19190 ^d	ND ^d	12000 ± 1700 ^d
	Week 3	65 ^a	ND ^d	126500 ± 56960 ^d	ND ^d	6500 ± 3100 ^d
	Week 4	85 ^a	ND ^d	139000 ± 20550 ^d	ND ^d	9250 ± 1130 ^d

Plants	Sample Time	Perchlorate Concentration (ppb) ± SD				Mass of Perchlorate in Plant System (ng) ± SD
		Unplanted Sand	Planted Sand	Leaves	Root	
H*:Milli-Q (25:75)	Week 0	108 ± 2 ^c	108 ± 2 ^c	NA	NA	10800 ± 250 ^c
	Week 0.5	107 ± 2 ^c	107 ± 2 ^c	NA	NA	10670 ± 170 ^c
	Week 1	99 ± 7 ^c	61 ± 18 ^b	127400 ± 31060 ^b	5500 ± 77770 ^b	9450 ± 3460 ^b
	Week 1.5	109 ^a	30 ± 13 ^d	201500 ± 22320 ^d	ND ^d	7550 ± 1600
	Week 2	108 ^a	25 ± 6 ^d	190600 ± 16030 ^d	9400 ± 18800 ^d	8000 ± 1460
	Week 3	72 ^a	2 ± 5 ^d	314400 ± 55700 ^d	ND ^d	12000 ± 2900
	Week 4	102 ^a	ND ^d	219100 ± 32950 ^d	ND ^d	10600 ± 1520
Milli-Q water	Week 0	110 ± 5 ^b	110 ± 5 ^b	NA	NA	5500 ± 230 ^b
	Week 1	85 ^a	16 ± 0.90 ^b	118100 ± 11280 ^b	ND ^b	4150 ± 520 ^b
	Week 2	98 ± 4 ^b	15.21 ^a	202400 ^a	ND ^a	1140 ^a
Hydrosol*	Week 0	106 ± 4 ^c	106 ± 4 ^c	NA	NA	10600 ± 420 ^c
	Week 0.5	96 ± 3 ^d	96 ± 3 ^d	NA	NA	9670 ± 1100 ^d
	Week 1	94 ± 5 ^b	55 ± 4 ^c	81600 ± 13550 ^c	ND ^c	7840 ± 990 ^c
	Week 1.5	128 ^a	41 ± 9 ^d	114400 ± 7190 ^d	19500 ± 39000 ^d	8120 ± 1890 ^d
	Week 2	85 ^a	57 ± 19 ^d	99900 ± 13830 ^d	33700 ± 67300 ^d	10140 ± 3770 ^d
	Week 3	96 ^a	22 ± 16 ^d	142100 ± 10500 ^d	ND ^d	10870 ± 3160 ^d
	Week 4	62 ± 3 ^b	6 ± 5 ^c	101700 ± 45350 ^c	ND ^c	7650 ± 2930 ^c
	Week 5	184 ^a	36 ± 22 ^b	119400 ± 61930 ^b	ND ^b	19150 ± 6770 ^b
	Week 6	164 ^a	47 ± 22 ^c	146600 ± 900 ^c	ND ^c	20100 ± 5580 ^c
	Week 7	238 ^a	45 ± 20 ^d	102100 ± 9300 ^d	ND ^d	17120 ± 4870 ^d
Week 8	149 ^a	20 ± 17 ^d	79800 ± 31300 ^d	ND ^d	14300 ± 3560 ^d	
Lettuce						
Hydrosol*	Week 0	69 ± 2 ^c	69 ± 2 ^c	NA	NA	3440 ± 80 ^c
	Week 1	70 ^a	72 ± 2 ^c	115700 ^a	ND ^a	5170 ± 70 ^c
	Week 2	82 ^a	68 ± 8 ^c	241300 ± 16640 ^d	ND ^a	5100 ± 890 ^d
	Week 3	55 ^a	72 ± 19 ^c	70300 ± 23200 ^c	ND ^c	6110 ± 1560 ^c
	Week 4	43 ^a	8 ± 11 ^c	753800 ± 32600 ^c	ND ^c	5200 ± 1640 ^c
	Week 5	79 ^a	ND ^c	20760 ± 6240 ^c	19250 ^a	2480 ± 450 ^c
	Week 6	71 ^a	ND ^c	31900 ± 14370 ^c	ND ^a	1690 ± 170 ^c
Soybean						
Hydrosol*	Week 0	12 ± 3 ^c	128 ± 3 ^c	NA	NA	6420 ± 150 ^c
	Week 1	114 ± 9 ^d	83 ± 8 ^d	13000 ± 6300 ^d	9300 ± 18600 ^d	5940 ± 910 ^d
	Week 2	77 ± 3 ^d	11 ± 10 ^d	15300 ± 3600 ^d	28800 ± 22600 ^d	3640 ± 1770 ^d
	Week 3	62 ± 5 ^d	8 ± 2 ^c	17800 ± 3950 ^c	ND ^c	2720 ± 480 ^c
	Week 4	67 ± 4 ^d	18 ± 16 ^d	14500 ± 3050 ^d	ND ^d	2800 ± 1550 ^d

NA = sample was not collected at this time point

ND = perchlorate was not detected

^a = sample size (n = 1)

^b = sample size (n = 2)

^c = sample size (n = 3)

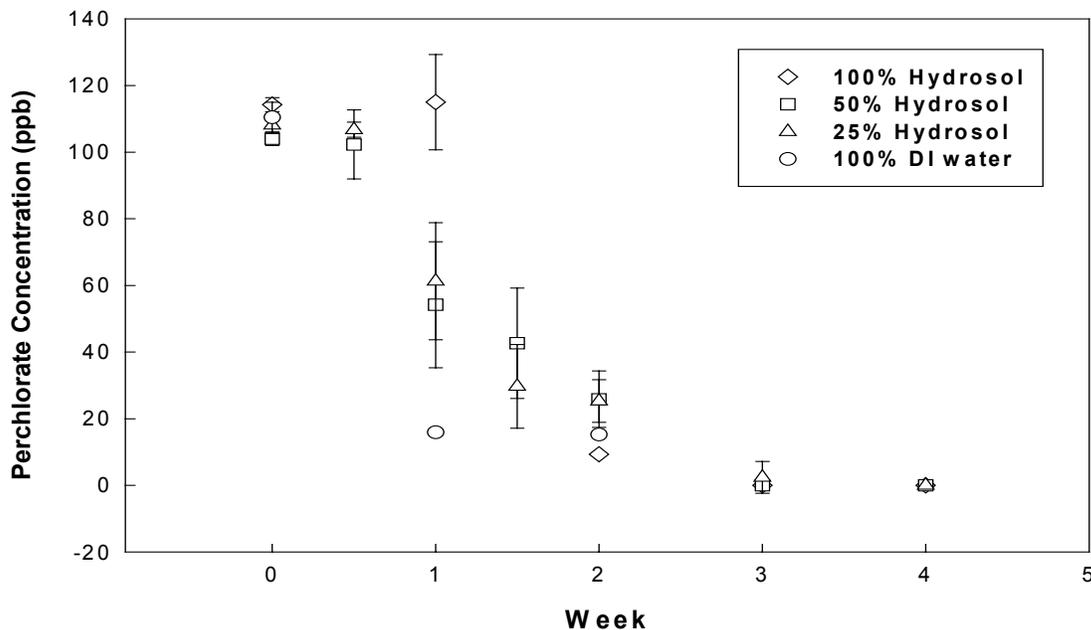
^d = sample size (n = 4)

^e = sample size (n = 5)

Week 0 indicates immediate extraction after perchlorate application.

*Diluted solution of Peters All Purpose Plant Food. Main components include nitrate, phosphate, magnesium, iron, copper, manganese, zinc, and molybdenum.

In this study, the potential effects of external nutrients were assessed by comparing perchlorate concentrations in planted sand, leaves, and roots grown in the presence of various ratios of Hydrosol to Milli-Q water. Evidence indicated that increased nutrient levels decreased the rate of perchlorate uptake into vegetation. For the cucumber experiments, sand concentrations of perchlorate in the presence of plants grown with 100% Hydrosol decreased the slowest, while those in 100% Milli-Q water decreased the fastest. Concentrations of perchlorate in the sand of experiments conducted with mixtures of Hydrosol and water decreased at a rate between those of pure Hydrosol and pure water (Figure 5-123). Consistent with the sand data, perchlorate uptake in leaves was the greatest when Hydrosol was not used to water the plants (Table 5-20). Although these perchlorate concentration differences in leaves may be due to decreased leaf mass because of a lack of nutrient(s), there is still an increased potential hazard for higher organisms due to the perchlorate concentration achieved in cucumber leaves. The differences in uptake could be due to certain nutrients in Hydrosol, especially nitrate, which may compete with perchlorate for uptake into plants; the presence of nitrate may essentially block perchlorate uptake. It is possible that perchlorate could only be taken up after most of the nitrate is removed. With fewer of these nutrients in sand, perchlorate was more available to accumulate in the plant. Due to the sampling protocol (weekly), perchlorate was rarely detected in roots. More frequent sampling of roots would be necessary to detect perchlorate before it translocates to leaves. A similar competition effect was observed in the soybean experiments, although in contrast to the cucumber experiments, the removal of perchlorate from sand occurred almost at the same rate for 100% Hydrosol and Hydrosol:Milli-Q water 50:50.



Error bars represent one standard deviation of the mean.

Figure 5-123
A Comparison among Sand Concentrations of Four Cucumber Perchlorate Uptake Experiments in the Presence of Varying Ratios of Hydrosol to Water

In the eight-week perchlorate uptake study, samples were respiked with 100 ppb perchlorate after Week 4. Concentrations in leaves peaked at Week 3 around 150 ppm and then peaked again at Week 6 (around 150 ppm) after the respike. It appears that the plants reached a maximum threshold of perchlorate, especially since the sand data indicated that there was still perchlorate available for uptake. This suggests that plants do not have the ability to hyperaccumulate perchlorate once a maximum burden in leaves is reached. At that point, the plant may begin to exude, transform, or transpire perchlorate. In the lettuce experiment, perchlorate concentrations in the leaves reached 750 ppm at Week 4 and then decreased to 20 ppm at Week 5, further supporting the idea of perchlorate exudation, transformation, or transpiration from leaves.

Although accumulation of perchlorate in leaves was observed in all plant species, there were significant differences in the ability of plants to take up perchlorate. Perchlorate concentrations in leaves were compared among cucumber, lettuce, and soybean experiments all treated identically (100% Hydrosol). Lettuce exhibited the highest accumulation of perchlorate at 750 ppm, followed by cucumber (41 ppm), and soybean (18 ppm). Lettuce showed a greater ability to accumulate perchlorate than the other two species. This difference is not completely explained by less plant mass for lettuce (the denominator in the concentration calculation) compared to the other two species. Significant differences ($P < 0.0001$) were observed for comparisons between perchlorate concentrations in lettuce vs. cucumber and soybean. There was no significant difference in perchlorate concentrations between cucumber and soybean.

Condensation was observed on Petri dishes covering the cups containing plants. This condensation was included in our analysis. Since water was only observed on Petri dishes covering the cups containing plants, the condensation is not due to evaporation from the sand, but rather transpiration from plant leaves. In the cucumber experiments, each water sample collected from the Petri dishes contained perchlorate, while in the soybean experiments, none of the water samples contained perchlorate. Further studies are needed to explain this process, but it appears that some plants can transpire perchlorate.

A perchlorate mass balance was calculated for each plant uptake experiment. In some experiments, there was an 80% loss of total mass by the end of the experiment. In other experiments, there was little perchlorate lost ($< 10\%$) during the study period (**Figure 5-122**). The primary reason for differences in mass balance among the experiments appears to be due to excessive rinsing of dried samples. After drying, leaves and roots were rinsed with Milli-Q water before extraction to remove perchlorate attached to the external surface of plants. Rinse water was analyzed for perchlorate. If there was perchlorate present in the rinsate, the plant was rinsed again until no perchlorate was found in the rinse water. In some of the first experiments, plants were rinsed several times before extraction. Because of its high water solubility, perchlorate may migrate from plants to water during rinsing which would remove perchlorate from plants and contribute to loss of total perchlorate mass. Therefore, experimental uncertainty was increased in data from the cucumber experiments in pure Hydrosol and pure water. Other factors that may also contribute to the loss of perchlorate include: (1) plant-mediated

transformation of perchlorate to chloride, and (2) expiration of perchlorate with transpiration water from plant leaves.

5.3.1.4 Discussion

Cucumber, lettuce, and soybean demonstrated their potential to take up perchlorate from contaminated sand. There was a significant perchlorate concentration burden for cucumber and lettuce. Results also suggest perchlorate depletion from sand and subsequent uptake into leaves is strongly influenced by the presence of nutrients in the sand. Plant species also affected perchlorate accumulation; the highest perchlorate concentration was achieved in lettuce. Plants in perchlorate-contaminated areas or crops grown with perchlorate-contaminated water represent a significant route of perchlorate exposure to higher organisms, including humans. However, external nutrients in soil appear to reduce the levels of perchlorate in plants. There was some evidence that perchlorate may be transpired by plants through evapotranspiration.

5.3.2 Perchlorate Uptake by Algae

5.3.2.1 Delta Areas

5.3.2.1.1 Introduction

Algae sampling was originally intended to be performed in the Lake Belton delta area to assess the possibility of algae and associated algae blooms taking up perchlorate and becoming a transport mechanism for perchlorate in this lake. Algae sampling was not performed in Lake Waco based on the project team's assumption that Lake Waco is a relatively well mixed, homogeneous environment and algae would not move via preferential pathways in this lake. Lake Waco's shallow configuration, alignment with the prevailing wind direction, and the presence of a mechanical aeration system provided the basis for this assumption. Additionally, higher perchlorate concentrations historically had been detected in the streams tributary to Lake Belton. Therefore, the team hypothesized that if algae did uptake perchlorate, detections of perchlorate in algae in Lake Belton would be more likely than in Lake Waco. This portion of the study was conducted as part of the Delta Areas Study. All the methodologies and protocol followed are detailed in the *Final Lake Belton and Lake Waco Delta Area Field Sampling Plan* (MWH, 2002c). Any deviations from the Field Sampling Plan are discussed further below.

5.3.2.1.2 Methodology

The algae sample collection activities were scheduled during the warmest time of the year when algae blooms would be expected to be prolific (i.e., during the summer). Algae sample collection activities were attempted on June 11, 2003 at all of the delta grid points established in Lake Belton as shown in **Figure 5-18** (Section 5.1.2).

A plankton net (Student Net) was used to collect bulk algae samples as shown on **Figure 5-124**. At each selected grid location, the plankton net was lowered to approximately 3 feet above the lake bottom and raised to the surface to capture algae in the sampler. Upon

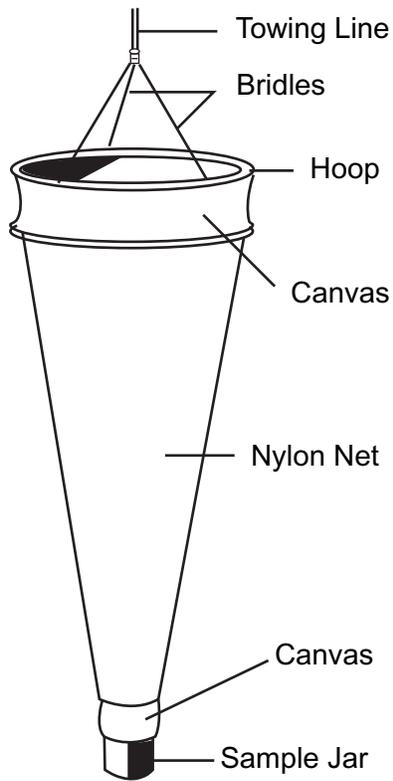
retrieval, the net was rinsed from the outside with distilled water to wash any algae that was adhering to the net into the cup at the bottom of the sampler. The water was allowed to drain from the cup and algae sample transferred to the appropriate sample container. The sampling procedure was attempted several times in order to obtain a sufficient volume of algae for the laboratory to perform the perchlorate analysis (i.e., 100 milliliters). However there were insufficient algae in the lake to collect any appreciable sample volume during this sampling event. Sampling activities were again attempted on August 21, 2003 both within and outside the delta areas. The locations within the delta area are shown in **Figure 5-18** (Section 5.1.2). The various locations outside the delta area where algae sampling was attempted are shown in **Figure 5-125**. The project team attempted to collect algae samples at a total of 80 locations within Lake Belton on two separate occasions and sufficient algae sample quantity was not available at any of the locations.

5.3.2.1.3 Data

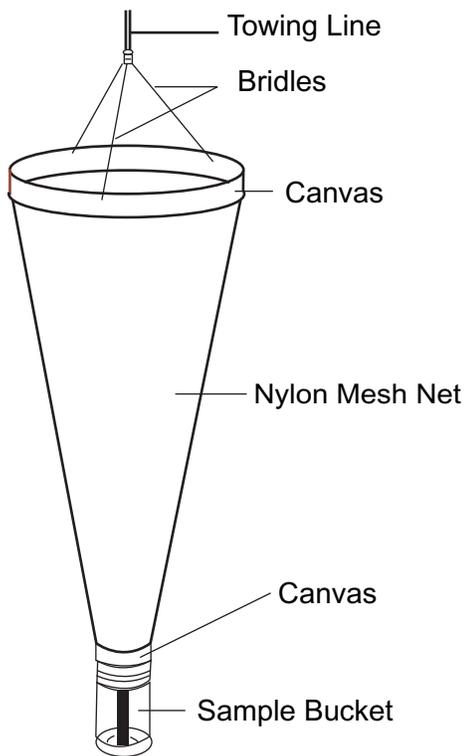
No data were generated as part of this study.

5.3.2.1.4 Discussion

Based on the two extensive attempts to collect algal samples in the summer of 2003, appreciable quantities of algae were not present in Lake Belton during this time. As a consequence, no data to evaluate the potential uptake of perchlorate by algae could be generated. It is not clear if the low occurrence of algae in Lake Belton was unique to these sampling events or is typically of the conditions in Lake Belton.



Student Net



Wisconsin Net

U.S. Army Corps of Engineers	
PROJECT:	
BOSQUE AND LEON RIVER WATERSHEDS STUDY	
DRAWING TITLE:	
PLANKTON NET SAMPLERS	
Sheet 1 of 1 sheets	
SCALE No Scale	FIGURE 5-124

Lake Belton

1:66,326

U.S. Army Corps of Engineers

Project:

**BOSQUE AND LEON RIVER
WATERSHEDS STUDY**

Drawing Title:

**LAKE BELTON ATTEMPTED
ALGAE SAMPLING LOCATIONS**

Sheet 1 of 1 Sheets

SCALE:
1:96,326

FIGURE:
5-125

Legend

- Attempted Algae Sampling Locations
- ▭ Delta Area

0 0.5 1 Miles



5.3.2.2 Stream Periphyton

5.3.2.2.1 Introduction

Routes of perchlorate exposure in aquatic environments was identified as a data gap related to conditions within the study area. For example, comparative analysis of perchlorate accumulation in fish and water from field-collected specimens indicates that perchlorate levels may be higher in fish than in water, but laboratory analysis indicates that perchlorate does not bioconcentrate in fish. This suggests that fish in the field are being exposed by some route(s) other than direct absorption from the water. One possible route of uptake in fish is through the food chain. In most stream ecosystems that have been studied, there is little phytoplankton (floating algae), unlike lakes and larger rivers (Minns, 1995). Thus, primary productivity is due to periphyton (attached algae) growing on rocks and other solid objects. One possible route of exposure for fish is through consumption of periphyton that have taken up and accumulated perchlorate from the water. However, there is little information on uptake of perchlorate in periphyton.

5.3.2.2.2 Methodology

Samples of periphyton (“algae”) were collected by grab sampling. Grab samples of algae were collected from at least 3 locations per sampling site, with each location at least 10 m apart. The sampling sites included Coryell Creek at Highway 84 (T36), Station Creek at Highway 107 (T23), the South Bosque at Highway 317 (T16), S Creek at Highway 317 (T15), Harris Creek at U.S. Highway 84 (T19) just west of McGregor, TX, and Wasp Creek at Highway 317 (T2). These sites are shown on **Figure 5-126**. At each location where algae were collected, three 60-ml samples of water were also taken for perchlorate analysis. Periphyton and water samples were kept on ice until transport back to the laboratory, then algae samples were frozen at -20 °C until analysis. Water samples were kept at 4 °C until analysis. Algae were thawed, desiccated under a fume hood, ground in a Warring blender, and extracted with water using accelerated solvent extraction. Algae extracts and water samples were analyzed for perchlorate using ion chromatography (**Appendix X**).

5.3.2.2.3 Data

The concentrations of perchlorate in water and algae samples collected are presented in **Table 5-21** and **Table 5-22**, respectively. Concentrations in algae are reported on a dry weight basis. In general, these data indicate that the concentration of perchlorate in algae may be equal to or greater than that found in the water. Bioconcentration factors (concentration in the algae/concentration in the water) for Harris Creek (T19), Station Creek (T23), and S Creek at Highway 317 (T15) were 8.4, 14.7, and 1.0, respectively. A bioconcentration factor could not be calculated for South Bosque at Highway 317 (T16) due to the fact that perchlorate was not detected in the water. However, historical data are available (**Appendix C**) and would suggest a high bioconcentration factor (> 100) for this location.

Table 5-21
Mean ± SD Perchlorate Concentration in Water Samples Collected at Various Streams near NWIRP in March, 2002

Location	Perchlorate (ppb)
Coryell Creek (T36)	ND
Wasp Creek (T2)	ND
Harris Creek at Highway 84 (T19)	24.3 ± 0.3
Station Creek at Highway 107 (T23)	21.9 ± 0.9
S Creek at Highway 317 (T15)	323 ± 4.1
South Bosque at Highway 317 (T16)	ND

n = 3. ND = not detected

Table 5-22
Perchlorate Concentration in Algae within the Waco Watershed

Location	Perchlorate (ppb)
Coryell Creek (T36)	ND
Wasp Creek (T2)	ND
Harris Creek at Highway 84 (T19)	204
Station Creek at Highway 107 (T23)	323
S Creek at Highway 317 (T15)	322
South Bosque at Highway 317 (T16)	376

Perchlorate concentrations are in ng/g (ppb) expressed as tissue wet weight
Samples collected in March 2002
ND = not detected.

5.3.2.2.4 Discussion

Smith et al (2001) demonstrated that terrestrial plants may bioconcentrate perchlorate thousands of times the concentration in the soil. However, such large bioconcentration factors were not found when comparing levels of perchlorate in water to tissue concentrations in emergent vegetation. However, up to now there have been no studies examining the bioconcentration potential of fully submerged aquatic plants. This is

significant because such epiphytic algae species may serve as the primary producers in small streams. The results presented above indicate that epiphytic algae may indeed bioconcentrate perchlorate, hence providing a pathway for food chain transport of perchlorate to higher trophic levels. This may be a direct source of exposure, because many species of fish are specialized for feeding on periphyton, which forms much or all of their diet. Such fish include stonerollers, carp, suckermouth minnows, some suckers and certain darters (Smith, 1979). Other fish are omnivorous, and consume many items, both plant and animal, but still regularly consume periphyton. These fish include many species of minnows and shiners (*Pimephales*, *Notropis*, *Cyprinella*, and *Notemegonus* spp.), several species of suckers, some sunfish, killifish, and mosquitofish (Smith, 1979). Fish may indirectly be exposed to perchlorate accumulated in algae through food chain transfer, because numerous invertebrates also feed upon algae and other periphyton (Wallace and Webster, 1996). These organisms include crayfish, isopods, amphipods, Chironomids (mayfly larvae), beetle larvae, and snails, all of which are fairly common in central Texas streams and are regularly consumed by at least some of the species of fish collected for analysis (Wallace and Webster, 1996).

The results reported above are notable for several reasons. First, they indicate that fish may be exposed to perchlorate levels much higher than that found in the water, so that monitoring water concentrations is insufficient to assess hazard, potential exposure or effects to fish. Second, these findings may help to explain results in Section 5.4.1, in which perchlorate was found in fish but not water; i.e., that food chain exposure via periphyton may contribute to fish body burdens. Third, it is notable that perchlorate was detected in the algae from South Bosque, but not in the water during this sampling period. However, low levels of perchlorate are routinely found in this river. This suggests that perchlorate concentrations in the South Bosque are highly variable, but that the concentrations in the algae is routinely higher, providing more opportunities for exposure than via uptake water. In fact if we use the highest concentration of perchlorate reported in this river in other sections of this report (i.e., 30 ppb reported in Section 5.4.2), then the minimum bioconcentration factor for algae in this stream is 12.5. If we take the average water concentration at this site reported in Section 5.4.2 (1.4 ppb), then the bioconcentration factor (BCF) for this site would be roughly 270 (assuming the concentration in algae remains relatively constant). This would suggest that the BCF for algae in the South Bosque was higher (perhaps by even one order of magnitude) than at any other site. Differences in BCFs between sites may reflect differences in algal species composition, temporal dynamics of perchlorate levels in the streams, or physiochemical properties of the streams themselves.

5.3.3 Perchlorate Uptake by Riparian Plants

5.3.3.1 Introduction

Perchlorate uptake by plants can be viewed as both a possible sequestration and perturbation process of perchlorate from sediments and streams. Terrestrial plants are capable of removing perchlorate from sediments and stream water and translocating it to leaves/fruits/nuts. Aquatic vegetation is capable of uptake directly from bulk stream water. Exposure of organisms through ingestion of plant material may depend on the

availability of perchlorate to the plant (seasonal and spatial), distribution within the plant (leaves/fruits/nuts), as well as the length of exposure. Sampling of plant matter from both terrestrial and aquatic vegetation will help to evaluate the overall potential for perchlorate uptake in plants as well as characterize the importance of this exposure pathway.

The fate of perchlorate in the environment can also be influenced by the potential re-release of perchlorate from vegetation after uptake. As plants senesce, vegetative tissues are returned to the soil/sediment. The return of perchlorate to the soil/sediment may be a beneficial process due to the movement of perchlorate from stable environments (aerated stream water or low carbon deeper sediments) to areas where rapid transformation can occur (top organic rich soil layers). On the other hand, uptake of perchlorate can also be viewed as another mechanism of exposure, especially if bioconcentration occurs in plant material. Measuring the concentration in leaf litter facilitated a better understanding of the recycling of perchlorate.

5.3.3.2 Methodology

Six locations surrounding NWIRP were sampled: Harris Creek at Highway 84 (HW84 Mainstream, T19), the spring on Oglesby Road (HW84 Sidestream, T18), Harris Creek at Highway 317 (HW317, T13), S Creek at Highway 317 (HW317/MN, T15), Station Creek at Highway 107 (HW107, T23), and the South Bosque at Mother Neff Road (Mother Neff Rd, T16). These sampling locations were distributed along three major creeks flowing away from the site, including Harris Creek, South Bosque River, and Station Creek. Detailed descriptions of these sampling sites are summarized in **Table 5-23** and shown on **Figure 5-127**.

Table 5-23
Description of Riparian Plant Sampling Locations

Site Name	Location	Samples Taken	Remarks
HW84 Mainstream (T19)	Harris Creek at HW84	Water, Aquatic plants	Water depth fluctuation of about 0.3-1.5 m yearly (Long-term sampling)
HW84 Sidestream (T18)	Spring on Oglesby Road	Water, Aquatic plants, Terrestrial plants	About 250 m length; relatively constant annual flowrate of about $4 \times 10^{-3} \text{ m}^3/\text{s}$ (Long-term sampling for water and aquatic and terrestrial plants along the stream; Seasonal sampling of tree leaves during the growing season along a defined reach of about 75 m long; Deciduous tree leaves were collected)
HW317 (T13)	Harris Creek at HW317	Water, Aquatic plants	Water depth fluctuation of about 0.3- 1.5 m yearly (Long-term sampling)
HW317/MN (T15)	S Creek at HW317	Water, Aquatic plants Terrestrial plants	Water depth fluctuation of about 0-0.6 m yearly (Long-term sampling)
Mother Neff Rd. (T16)	South Bosque River at Mother Neff Road	Water, Aquatic plants	(Short-term sampling)
HW107 (T23)	Station Creek at HW107	Water, Aquatic plants	(Short-term sampling)

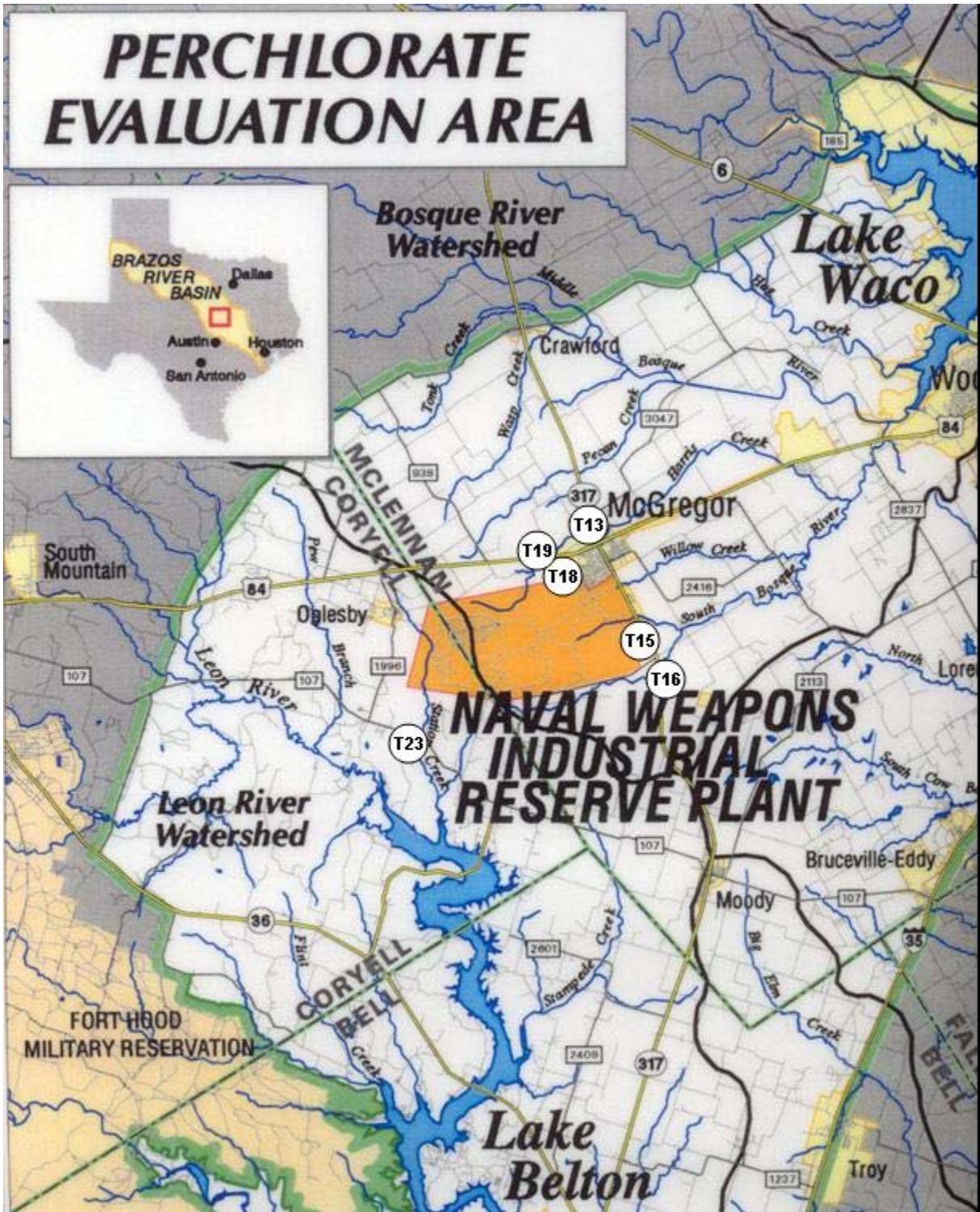


Figure 5-127
Map of Study Area Illustrating the Approximate Locations where Riparian Plant Samples Were Collected

Generally, water and vegetation sampling were conducted from June 2001 through November 2002, at an interval of every one to two months. Initially, short-term sampling was conducted for all the sites including Mother Neff Rd (T16) and HW107 (T23). Then, over a one-year period sampling was conducted to focus on the remaining sites. Two dominant aquatic plants, smartweed (*Polygonum* sp.) and watercress (*Rorippa* sp.), were collected whenever they were present in the streams.

In addition, starting from April, 2002, seasonal tree leaf sampling was conducted for more than sixty trees, including ash (*Fraxinus greggii*), china-berry (*Malia azedarach*), elm (*Ulmus parvifolia*), mulberry (*Broussonetia papyrifera*), and hackberry (*Celtis laevigata*), which grew on a forestland (approximately 75 m long and 25 m) wide) of HW84 Sidestream (T18). Trees along the reach were tagged and their locations were recorded. Tree leaves were sampled every two months during the growing period of macrophytes (from budding to leaf drop). At the end of October, 2002, four leaf litter traps (1.2 m length x 1.2 m width) were deployed at four locations on this forestland to collect the deciduous leaves. Deciduous leaves were collected after all the leaves had fallen from the trees. Distribution of trees sampled and the locations of litter traps are presented in **Figure 5-128**.

Bulk water samples were taken by using 30 ml plastic sample vials whenever there was water in the streams. Aquatic plants were rinsed with deionized water and then stored in plastic zip bags. Leaves of terrestrial plants were sampled from different locations and placed into pre-labeled bags. All samples were put on ice and transferred to the laboratory. Aquatic and leaf samples were mixed individually in the laboratory and then sub-samples were taken for further analysis.

Water samples were filtered (0.45 μm) and analyzed for perchlorate directly. Aquatic and terrestrial plants were extracted following the general method of Ellington and Evans (2000). Aquatic plants were rinsed once again with deionized water and blotted dry with paper towels. Aquatic and terrestrial plant tissues were cut into 0.5-1 cm pieces and dried in the refrigerator for several days. Collected plant material was subsampled and about 0.5 g of dried plant tissues (dry weight) were weighed and placed in 30 mL centrifuge tubes. Thirty mL of deionized water were added. In some cases duplicate or triplicate analysis were run on a given collection of plant material. However, in most cases only one sample from a given plant collection was evaluated. The centrifuge tubes were then tightly capped and placed in a boiling water bath for 1 hour, cooled to room temperature, and then placed in a 3 °C refrigerator for one day. Samples were shaken regularly. Five ml of supernatant out of the total 30 ml was collected with a pipette and then added to 5 g of pre-cleaned aluminum oxide adsorbent (Al_2O_3 , Aldrich, surface area, 155 m^2/g ; 150 mesh) for 1 day at 3 °C. Samples were filtered with prefilters (Millipore Corporation) and 0.45 μm IC syringe filters (Pall Corporation), diluted with 18 M Ω water (1:5), and analyzed for perchlorate (**Appendix X**).

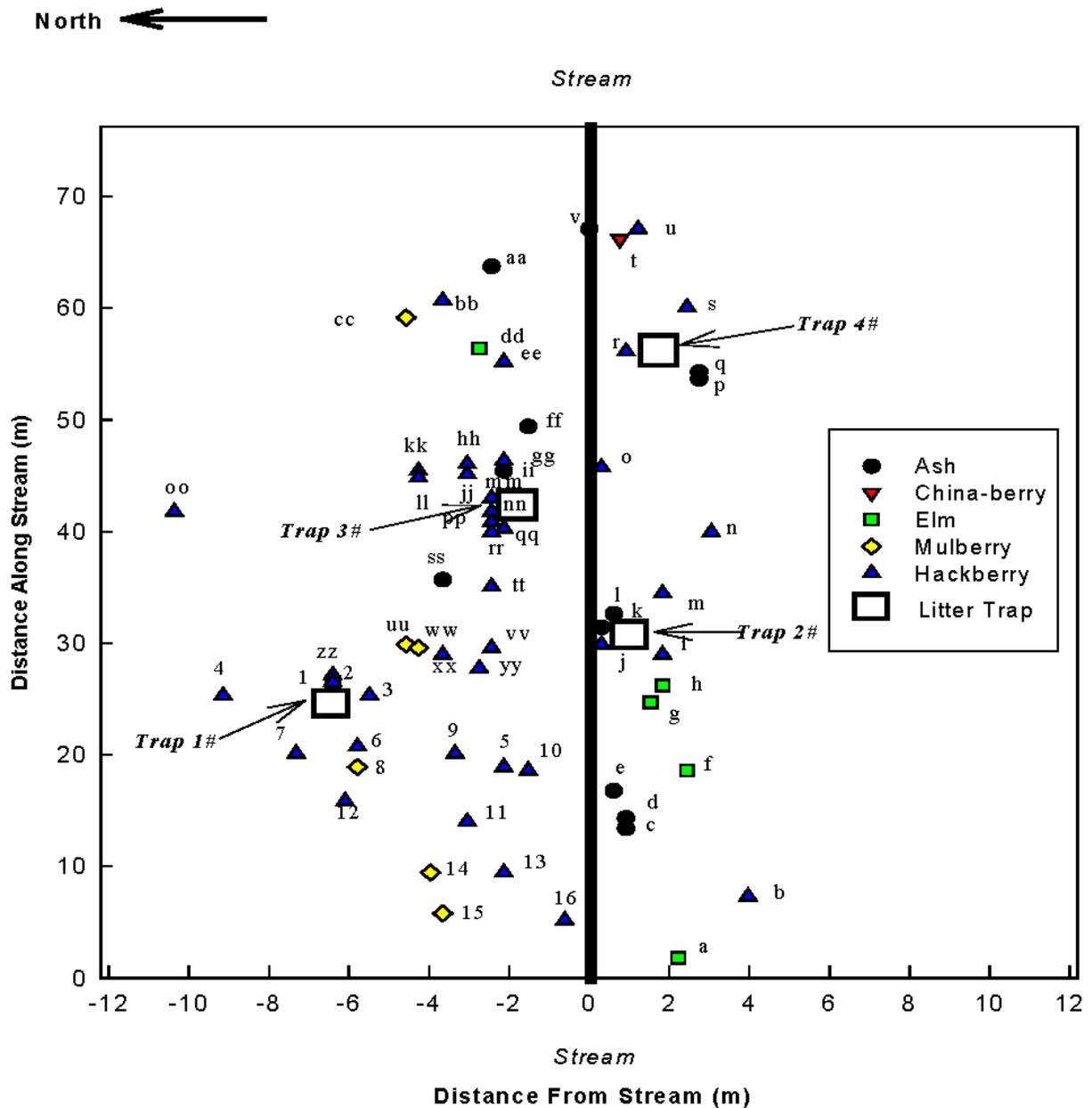


Figure 5-128
Distribution of Trees Sampled along a Defined Reach of HW84 Sidestream (T18)
(Not to Scale). All trees Were Tagged with Different Letters or Numbers.

5.3.3.3 Data

5.3.3.3.1 Perchlorate Concentration in Surface Water

Perchlorate concentration in surface water was site-specific and temporally variable (**Figure 5-129**). The highest perchlorate concentration (up to 536 ppb) was observed at HW317/MN (T15). Compared to the other three sites, the HW317/MN location had the highest ClO_4^- concentration in the stream, probably because this location is close to an explosives disposal area within NWIRP (Hare, 2000). Perchlorate concentrations at HW84 Sidestream (T18) were relatively stable from January 2002 to October 2002 (20-40 ppb), but a peak of 123 ppb concentration was observed in October, 2001. HW84 sidestream's initial source is a spring. Both HW84 Sidestream and HW317/MN consistently contained ClO_4^- . Considerable fluctuation in perchlorate concentrations was observed at HW84 Mainstream (T19), but perchlorate was detected in most months (11 out of 16 samples). Perchlorate concentrations were below the detection limit (4 ppb) in August and September 2001, and August 2002, which was linked with rapid bacterial activity in natural wetland habitats at higher temperature. Significant variation in ClO_4^- concentrations was also observed at HW317. It was evident that both HW84 Mainstream and HW317 (T13) intermittently received influxes of ClO_4^- . The variation of ClO_4^- concentration may also have been caused by seasonal flowrate fluctuation in the streams due to different temporal evaporation and precipitation rates. On April 6th, 2002, surface water samples were collected at HW84 Mainstream and HW317 immediately after a heavy thunderstorm event and very low perchlorate concentrations were observed. HW317 was located approximately 3 km downstream from HW84 Mainstream and exhibited a similar temporal trend in ClO_4^- concentrations.

5.3.3.3.2 Perchlorate Uptake in Aquatic Plants

Overall, ClO_4^- uptake in smartweed was observed at all the selected sites where ClO_4^- was detected in surface water, except HW84 Mainstream (T19) (**Table 5-24**). Although the ClO_4^- concentration in water averaged 12 ppb at HW84 Mainstream (**Table 5-24**), ClO_4^- may have been depleted in sediment pore water due to microbial degradation in the sediments when smartweed samples were collected, so that ClO_4^- uptake was not observed. A previous kinetics study (5.2.3) demonstrated the rapid microbial degradation in sediment of HW84 Mainstream (intrinsic ClO_4^- degradation rate constant $k = 0.14 \text{ d}^{-1}$) (Tan et al., 2004). Sediment pore water concentrations monitored by in-situ dialysis samplers from another study indicated that ClO_4^- was not present in sediment at HW84 Mainstream (Tan, 2003), which supports this hypothesis. In addition, higher uptake in smartweed was generally observed at sites with higher average ClO_4^- surface water concentrations. Average ClO_4^- uptake in smartweed ranged from 9,140 to 40,600 ppb ($\mu\text{g}/\text{kg}$ DW) for sites HW317/MN, HW84 Sidestream, and HW107, much higher than the range observed at HW84 Mainstream, HW317, and Mother Neff Rd. (< D.L. of 3,180 ppb). Watercress was collected from sites HW84 Sidestream, HW84 Mainstream, and HW107. Average perchlorate uptake in watercress ranged from 625 to 4,860 ppb ($\mu\text{g}/\text{kg}$ DW) (**Table 5-24**).

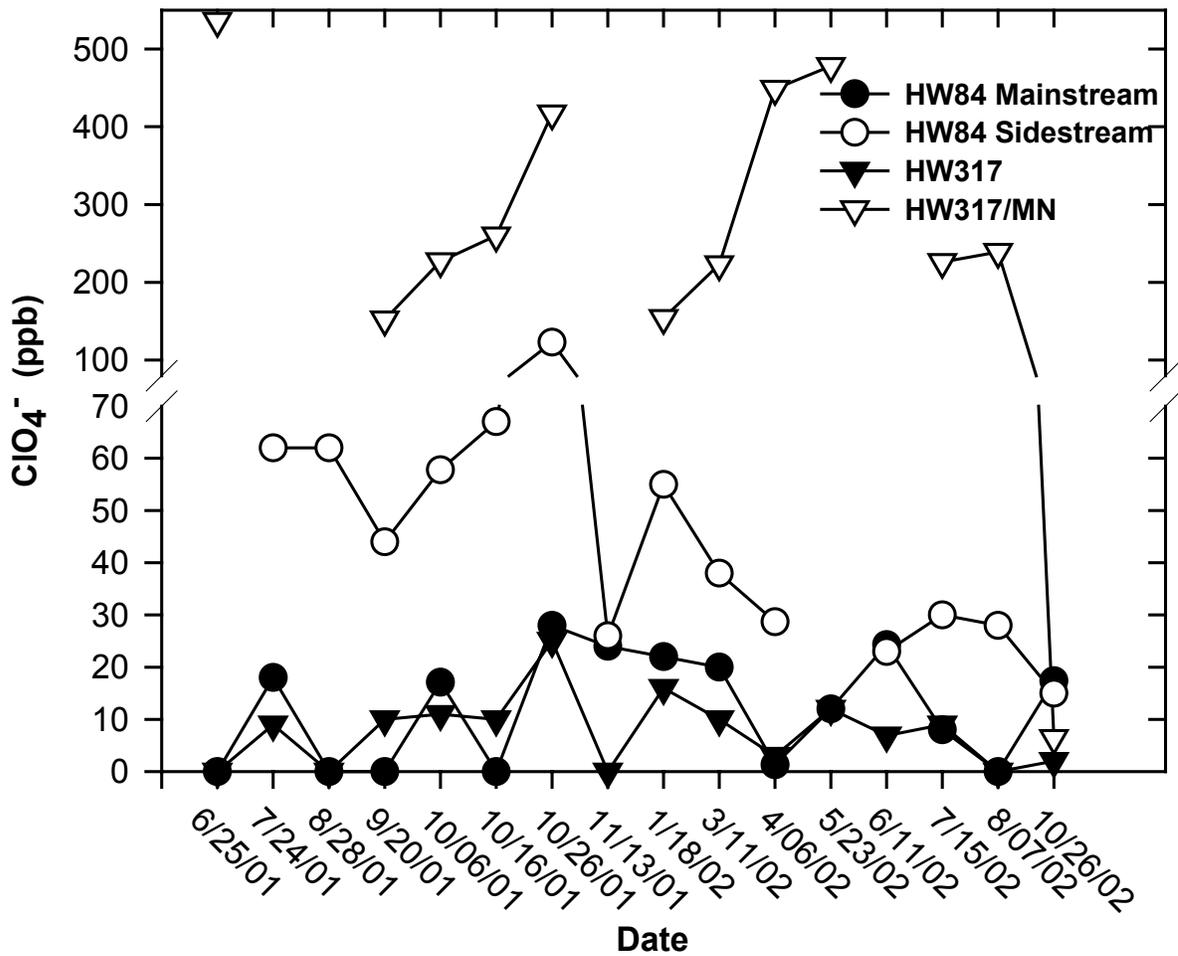


Figure 5-129
Perchlorate Concentrations in Surface Water at Different Stream Sites from June 2001 through October 2002

(Points not connected by solid lines means data missing because water samples were not taken.)

Table 5-24
Perchlorate Concentrations in Streams and Aquatic and Terrestrial Plants at
Multiple Sites Surrounding NWIRP

Site	Matrix/Species	Concentration (ppb)				Sample Size (n)
		Min.	Average	Max.	SD	
HW84 Sidestream (T18)	Water	15	47	123	28	12
	Smartweed	5850	9240	12200	2650	4
	Watercress	< D.L. [†]	5043	13900	3750	22
	Ash (leaf)	< D.L.	2330	17000	3510	24
	China-berry (leaf)	631	5040	9440	6230	2
	China-berry (fruit)	< D.L.	< D.L.	< D.L.	-	1
	Elm (leaf)	< D.L.	2230	14200	3880	18
	Mulberry (leaf)	< D.L.	1310	6660	1720	21
	Mulberry (fruit)	< D.L.	467	934	661	2
	Hackberry (leaf)	< D.L.	4690	48200	10100	111
	Willow (leaf)	1580	1580	1580	0	1
HW84 Mainstream (T19)	Water	< D.L. [‡]	12	28	11	15
	Smartweed	< D.L.	< D.L.	< D.L.	-	3
	Watercress	2630	4860	7090	3150	2
HW317 (T13)	Water	< D.L.	8	25	7	13
	Smartweed	< D.L.	3180	10500	4300	5
	Watercress	< D.L.	625	1560	705	6
HW317/MN (T15)	Water	6	281	536	157	12
	Smartweed	20700	40600	61600	20500	3
	Elm (leaf)	677	699	722	32	2
	Hackberry (leaf)	< D.L.	1300	2100	1140	3
	Willow (leaf)	< D.L.	6590	24300	8780	11
Mother Neff Road (T16)	Water	< D.L.	< D.L.	< D.L.	-	2
	Smartweed	< D.L.	< D.L.	< D.L.	-	2
HW107 (T23)	Water	15	17	20	3	2
	Smartweed	4280	9140	14000	6880	2

All samples were collected from June 2001 through October 2002

[†]D.L. (method detection limit) of ClO₄⁻ in water is 1 ppb.

[‡]D.L. (method detection limit) of ClO₄⁻ in plants is 300 ppb (μg/kg DW).

Perchlorate uptake in aquatic plants was regressed against ClO₄⁻ bulk water concentration in streams. There was a significant linear relationship between bulk water ClO₄⁻ concentration and plant uptake in smartweed (n = 19; R² = 0.7410; P < 0.0001) and watercress (n = 30; R² = 0.6022; P < 0.0001) (**Figure 5-130** and **Figure 5-131**). Results indicated that plant uptake of ClO₄⁻ from streams was substantial with dry leaf concentrations up to 2 orders of magnitude greater than bulk water concentrations. From the slope, the plant bio-concentration factor (BCF) on the basis of plant dry weight was estimated to be 164 for smartweed and 267 for watercress, respectively. A laboratory study by Susarla et al. (2000) indicated that as high as 456 mg/kg ClO₄⁻ (based on plant fresh weight) uptake was taken up by smartweed exposed to 20 ppm ClO₄⁻ during a ten-day exposure experiment. Assuming 70% water content in smartweed, the bio-

concentration factor (BCF) of Susarla's study (2000) was estimated to be about 76 (on the basis of plant dry weight), which is slightly lower than our results. Ten days may not be sufficient to achieve equilibrium of perchlorate uptake and the concentration tested is unrealistic compared to most surface water concentrations of perchlorate.

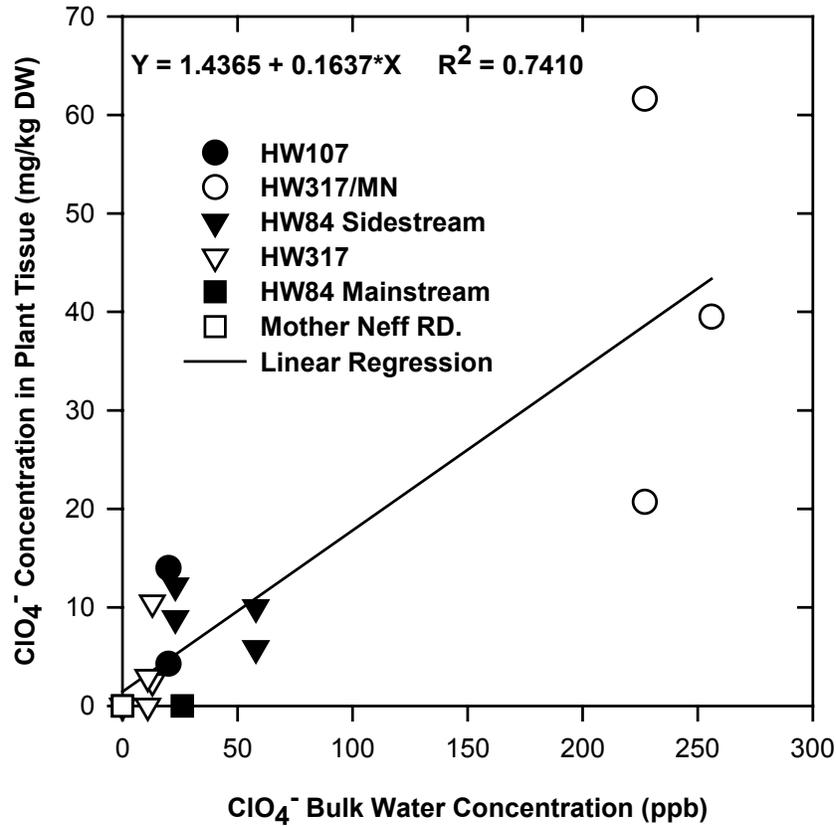


Figure 5-130
Relationship between ClO₄⁻ Bulk Water Concentration and Plant Tissue Concentration at Different Sampling Locations for Smartweed

(n = 19, P value of regression < 0.0001)

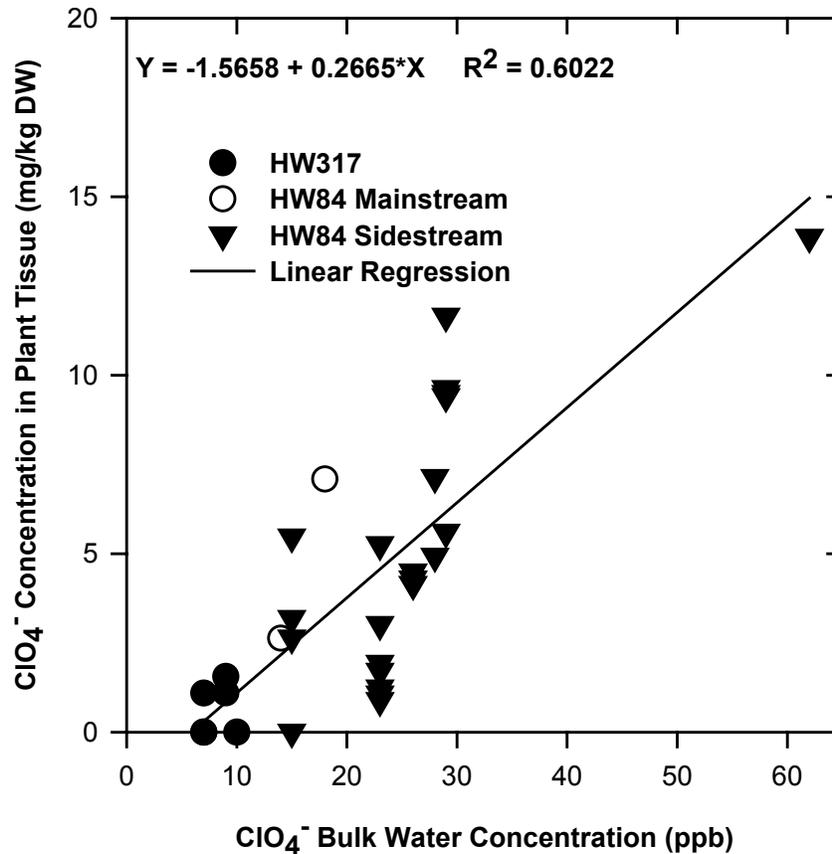


Figure 5-131
Relationship between ClO₄⁻ Bulk Water Concentration and Plant Tissue Concentration at Different Sampling Locations for Watercress

(n = 30, P value of regression < 0.0001)

Compared to watercress, smartweed has a more dense and longer root system that can extend deep into the sediment, so smartweed may take up perchlorate primarily from sediment pore water. Watercress is a floating aquatic plant with short roots that can take up perchlorate directly from surface water and shallower sediment pore water. Thus, the BCF estimated on the basis of the bulk surface water perchlorate concentration instead of the pore water concentration may underestimate the uptake potential of smartweed, because the pore water concentration is typically much lower than the bulk water concentration due to bacterial degradation in sediments. Because the actual sediment pore water concentrations at the locations where smartweed samples were collected were not available, bulk water concentrations were used to estimate the BCF in smartweed. Therefore, smartweed might have a higher perchlorate uptake potential than watercress even if the BCF of smartweed estimated on the basis of the bulk water concentration is lower than that of watercress. As a result, observed average perchlorate uptake in smartweed at HW84 Sidestream and HW317 was relatively higher than that of watercress (Table 5-24). At HW84 Mainstream, there was no perchlorate uptake in smartweed, due to the absence of perchlorate in the sediment pore water. In contrast, there was 4,860 ppb

in watercress because perchlorate was present in the bulk water for uptake stressing the importance of spatial exposure (**Table 5-24** and **Figure 5-131**).

5.3.3.3.3 Perchlorate Uptake Potential in Different Species of Terrestrial Plants

Terrestrial plant leaf samples were collected from two sites with consistent perchlorate concentrations, HW84 Sidestream and HW317/MN. Perchlorate uptake in all trees within 5 m from the stream was averaged to evaluate overall perchlorate uptake potential of different species (**Table 5-24**). All tree species investigated (ash, china-berry, elm, mulberry, hackberry, and willow) showed significant potential for perchlorate uptake, although perchlorate uptake was variable and species dependent. Highest perchlorate uptake was observed in hackberry (48,200 ppb), willow (24,300 ppb), and elm (14,200 ppb) (**Table 5-24**), implying these species may be good candidates for phytoremediation. In addition, perchlorate was preferentially accumulated in the leaf of china-berry and mulberry trees (average 5,040 ppb and 1,310 ppb, respectively) rather than the fruit (0 ppb and 467 ppb, respectively) (**Table 5-24**), implying that perchlorate was selectively partitioned in these plants.

5.3.3.3.4 Seasonal Uptake of Perchlorate in Terrestrial Plants

Sampling of tree leaves during the growing season at the HW84 Sidestream site indicated that perchlorate uptake in terrestrial plants was temporally and spatially variable and dependent on exposure duration. Generally, terrestrial plants up to 9 m from the stream accumulated significant concentrations of perchlorate (**Figure 5-132**). Trees located closer to the stream seemed to have higher perchlorate uptake, but in some cases trees located farther away from the stream also exhibited high perchlorate uptake. This may be caused by the heterogeneous distribution and penetration of root systems of trees. Some trees located further away from the stream may have widely-extended root systems to absorb water from the stream. For most trees, lower leaf ClO_4^- concentrations were observed in April and June, 2002, and the highest leaf tissue concentrations were observed late (August and October, 2002) in the growing cycle (**Figure 5-132**). Seasonal sampling of a willow (*Salix nigra*) tree from another site (HW317/MN) also indicated that perchlorate uptake in leaf tissue was seasonally variable (**Figure 5-133**). Perchlorate uptake in the willow tree increased with progression through the growth cycle. In April and May, 2002, there was no perchlorate uptake observed in new buds. In June and August, 2002, perchlorate uptake in leaves was 150 ± 210 ppb and $1,200 \pm 250$ ppb, respectively. The highest uptake in leaves was observed late in the growing cycle ($15,800 \pm 8,200$ ppb and $14,600 \pm 11,400$ ppb for October, 2001 and October, 2002, respectively).

The reason that perchlorate concentrations in some trees showed declining trends in October, 2002 compared to that in August, 2002 is not clear (**Figure 5-132**). Phytodegradation may contribute to a partial decrease of perchlorate in plant tissues, but this process normally occurs relatively slowly (Nzengung et al., 1999; Aken and Schnoor, 2002). Nzengung et al. (1999) estimated phytodegradation accounted for approximately 11% ClO_4^- loss in a 26 day laboratory growing experiment using willow trees.

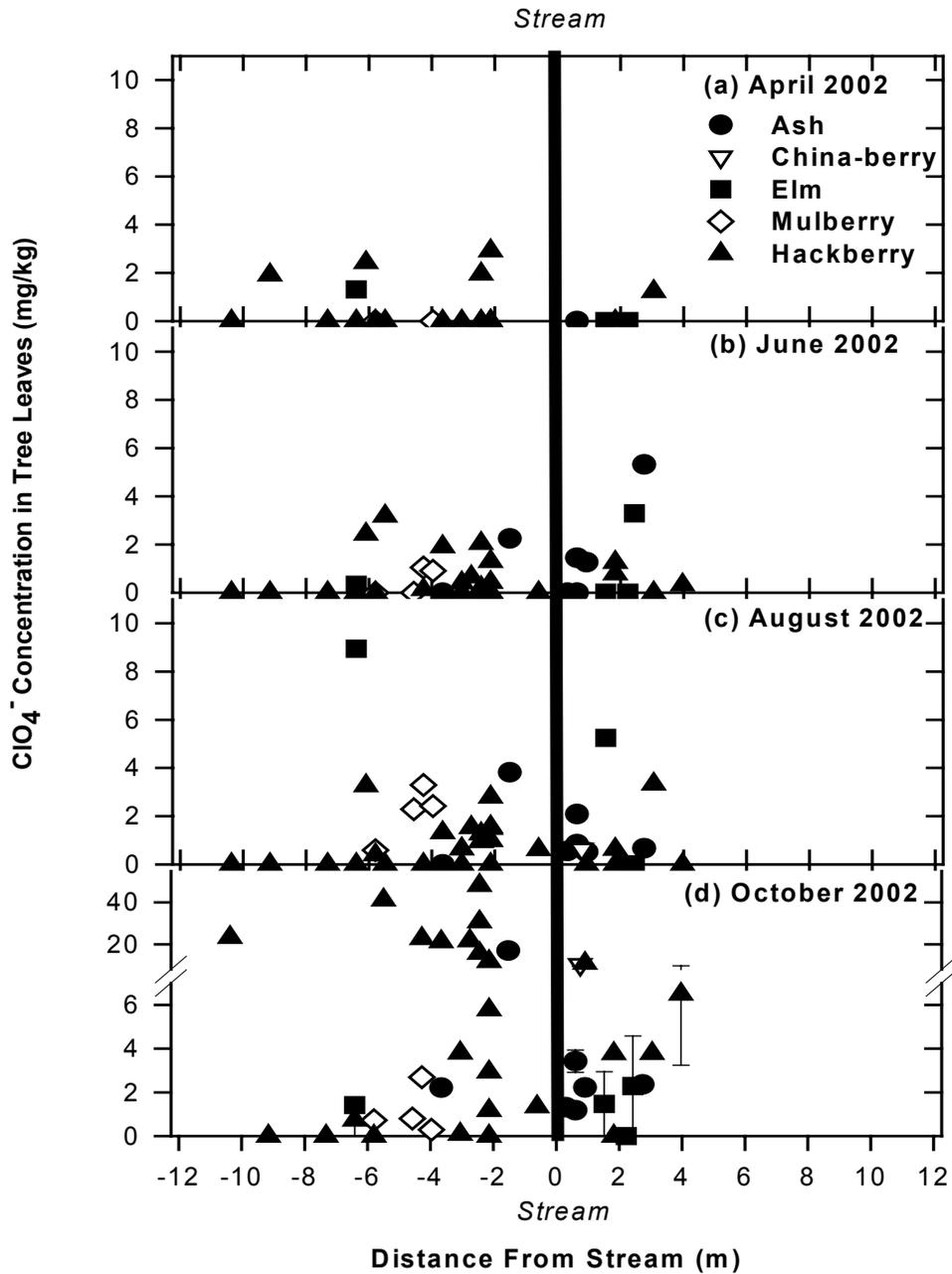


Figure 5-132
Perchlorate Seasonal Distribution in Tree Leaves along a Defined Reach of HW84 Sidestream (T18)

(Standard error bars represent the standard deviation of duplicate (n = 2) for some trees sampled in October 2002.)

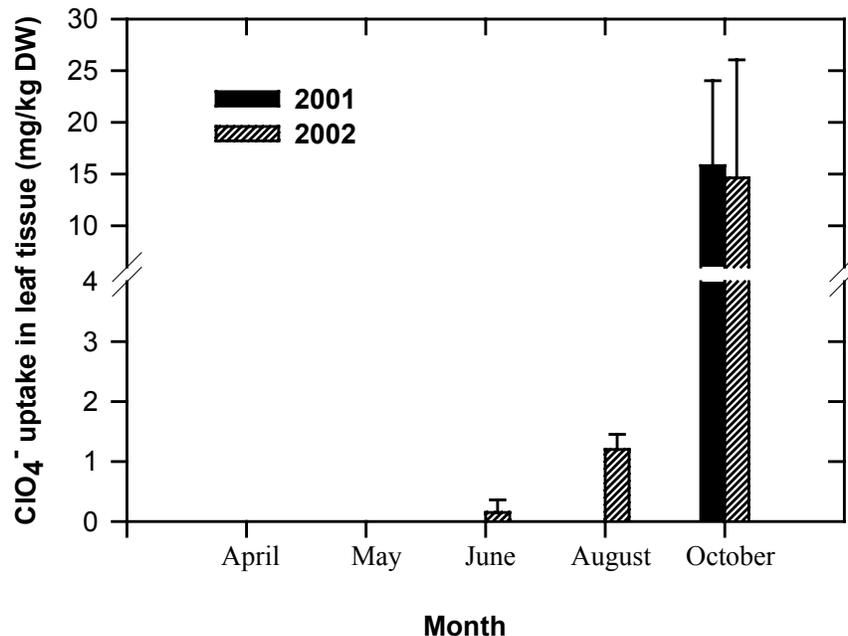


Figure 5-133

Seasonal Variation of ClO₄⁻ Uptake in a Willow Tree Located at HW317/MN (T15)

(Tree leaves were sampled from October, 2001 to October, 2002. Standard error bars represent the standard deviation of duplicate samples (n = 2).)

Another hypothesis is that the availability of other ClO₄⁻ sources, such as contaminated soil and groundwater, may also affect the uptake and distribution of ClO₄⁻ in terrestrial plants. However, information obtained from the HW84 Sidestream site suggests that terrestrial plants grown on this site absorb ClO₄⁻ mainly from the stream. In general, ClO₄⁻ was not detected in soil samples up to a depth of 60 cm beneath the surface. Only 1 out of 11 soil samples had a ClO₄⁻ concentration of 0.03 mg/kg DW. Results obtained suggested that soils near HW84 Sidestream were not contaminated by ClO₄⁻. The boring log of a groundwater monitoring well OFFHC-14 located near HW84 Sidestream was composed of brown silty clay with limestone cobbles (0 - 1.2 m), slightly soft and moist clay (1.2 - 2.3 m), hard and silty argillaceous limestone/shale (2.3 - 3.0 m), and dry dark gray shale (3.0 - 5.2 m). Perchlorate concentrations were below the detection limit (4 ppb) in groundwater from OFFHC-14 from May 2000 to July 2001 (Personal communication with MWH Global in July 2003). This information suggested that trees at this site take up ClO₄⁻ mainly from the stream instead of from groundwater sources. An attempt was made to correlate tree size with ClO₄⁻ uptake, but no correlation was found. Tree size may affect ClO₄⁻ uptake in case of vertical contaminant source present, i.e. contaminated groundwater or soil, because bigger and taller trees tend to have a deeper root extension. However, in this case, ClO₄⁻ uptake in terrestrial plants was mainly influenced by the distance to the stream, since only the horizontal ClO₄⁻ source (stream) was available for uptake. On the other hand, large-trunk-size trees with a wide root

system could also have a relatively high ClO_4^- uptake even if they were located farther away from the contaminated stream.

5.3.3.3.5 Perchlorate in Leaf Litter Traps

Perchlorate concentrations in deciduous tree leaves collected from four litter traps (layout shown above in **Figure 5-128**) in January, 2003 were compared with concentrations in live tree leaves sampled prior to leaf drop in October, 2002. Average concentration of trees located in the proximity of litter traps (within a radius of 1.5 m away from individual litter traps) was considered to be typical ClO_4^- concentration in the late growth cycle. Generally, average ClO_4^- leaf tissue concentrations prior to leaf drop were higher than that in tree leaves after leaf drop for individual litter traps (**Table 5-25**). It was statistically significant (t-test, $P < 0.05$) that ClO_4^- concentrations in deciduous tree leaves in litter trap 3 and 4 were lower than those in live leaves prior to leaf drop (**Table 5-25**). However, in some cases (e.g., in litter trap 1 and 2), according to the statistical analysis (t-test), there was no significant difference in ClO_4^- concentration before and after leaf drop ($P > 0.05$).

Table 5-25
Perchlorate Concentration in Tree Leaves before and after Leaf Drop

Litter Trap	ClO_4^- Concentration in tree leaves (mg/kg DW)	
	Before leaf drop [†]	After leaf drop [‡]
1 #	0.71 ± 1.00 [§]	< D.L. (0.3)
2 #	2.16 ± 1.39	1.20 ± 0.28
3 #	4.79 ± 1.39	1.75 ± 0.26
4 #	10.93 ± 0.0	2.83 ± 0.21

[†] ClO_4^- uptake in live tree leaves located within a radius of 1.5 m away from individual litter traps (sampled in October, 2002)

[‡] ClO_4^- concentration in deciduous tree leaves collected in individual litter traps (sampled in January, 2003)

[§] Average \pm standard error (n = 2).

The decrease of perchlorate concentration in deciduous leaves was most likely associated with leaching, rainfall events, and microbial degradation. Perchlorate from plants may be released into the environment, suggesting that plants may serve as source (re-release) and sink (uptake) of perchlorate. The selection of suitable plants may become a key factor in successful phytoremediation. The potential of evergreen plants to remediate perchlorate needs to be considered. If deciduous plants are to be used in phytoremediation of perchlorate, harvest and subsequent disposal after the plants hyperaccumulate perchlorate may be necessary.

5.3.3.4 Discussion

This research elucidated the fate of ClO_4^- in macrophytes in natural systems using the NWIRP site as a case study. Perchlorate concentrations in surface waters at multiple streams were temporally variable, depending on numerous factors such as contamination source, fluctuation of flowrate, and bacterial degradation. Significant ClO_4^- uptake was observed in smartweed and watercress (BCFs of 163 and 266, respectively) that dominated the natural wetland habitat. Perchlorate uptake in leaves of terrestrial plants was dependent on numerous factors, such as plant species, accessibility to the ClO_4^-

source, contamination levels in soils and groundwater, as well as exposure duration. Perchlorate taken up by trees may re-release to the environment after uptake. Results indicated that terrestrial plants at this site mainly take up perchlorate from streams rather than from groundwater. However, at some sites with a shallow groundwater table, if terrestrial plants absorb and accumulate ClO_4^- from groundwater and the BCF of a specific plant species is known, terrestrial plants may serve as another alternative to monitor ClO_4^- contamination in groundwater by simply determining the ClO_4^- uptake in terrestrial plants overlying the groundwater table. Tree size may become an important factor to affect the uptake of ClO_4^- , since bigger and taller trees tend to have a deeper and more dense root system capable of reaching the groundwater. Thus, terrestrial plants could become a useful bio-monitoring tool as a supplement to costly groundwater monitoring wells. Information obtained will be helpful to tailor a site-specific design and management protocol of phytoremediation to remediate ClO_4^- contamination in other similar sites with non-point ClO_4^- contamination sources.

5.3.4 Market Basket Survey

5.3.4.1 Introduction

An important question in the study area is the potential for human exposure to perchlorate through the consumption of contaminated food. The use of irrigation water (surface or groundwater), which may be contaminated with perchlorate, to supply water for gardens is a relevant scenario in the study area. Under this scenario it is critical to establish the relationship between perchlorate concentrations in irrigation water and perchlorate concentrations in edible vegetation. In order to establish this relationship and determine the exposure potential for perchlorate to humans, edible plants or their surrogates were sampled from several locations within the study area. In addition, various vegetable samples from other locations were collected to complete the data set.

5.3.4.2 Methodology

Samples (vegetation, soil, and water) for this study were obtained by making personal contact with landowners and/or home owners along drainages within the study area. Descriptions of the sampled sites, along with the types of samples collected are provided below. The locations of these sites are indicated on **Figure 5-134**.

5.3.4.2.1 Harris Creek at Windsor Road (T30)

This location was near a longitudinal stream sampling station that was part of the overall project. The homeowner at this location allowed us to collect samples from the garden. The home owner indicated that water from Harris Creek was used to irrigate the garden. Water, soil, and potatoes were collected from this location.

5.3.4.2.2 Station Creek (T20-T23)

We were unable to identify a property owner or home owner along Station Creek (from the Texas A&M property near NWIRP to the Leon River) that had a garden. However, we collected samples from two locations along Station Creek: (1) at the Texas A&M property, and (2) near Highway 107. Samples collected included water, leafy vegetation (including sunflowers), berries and nuts.

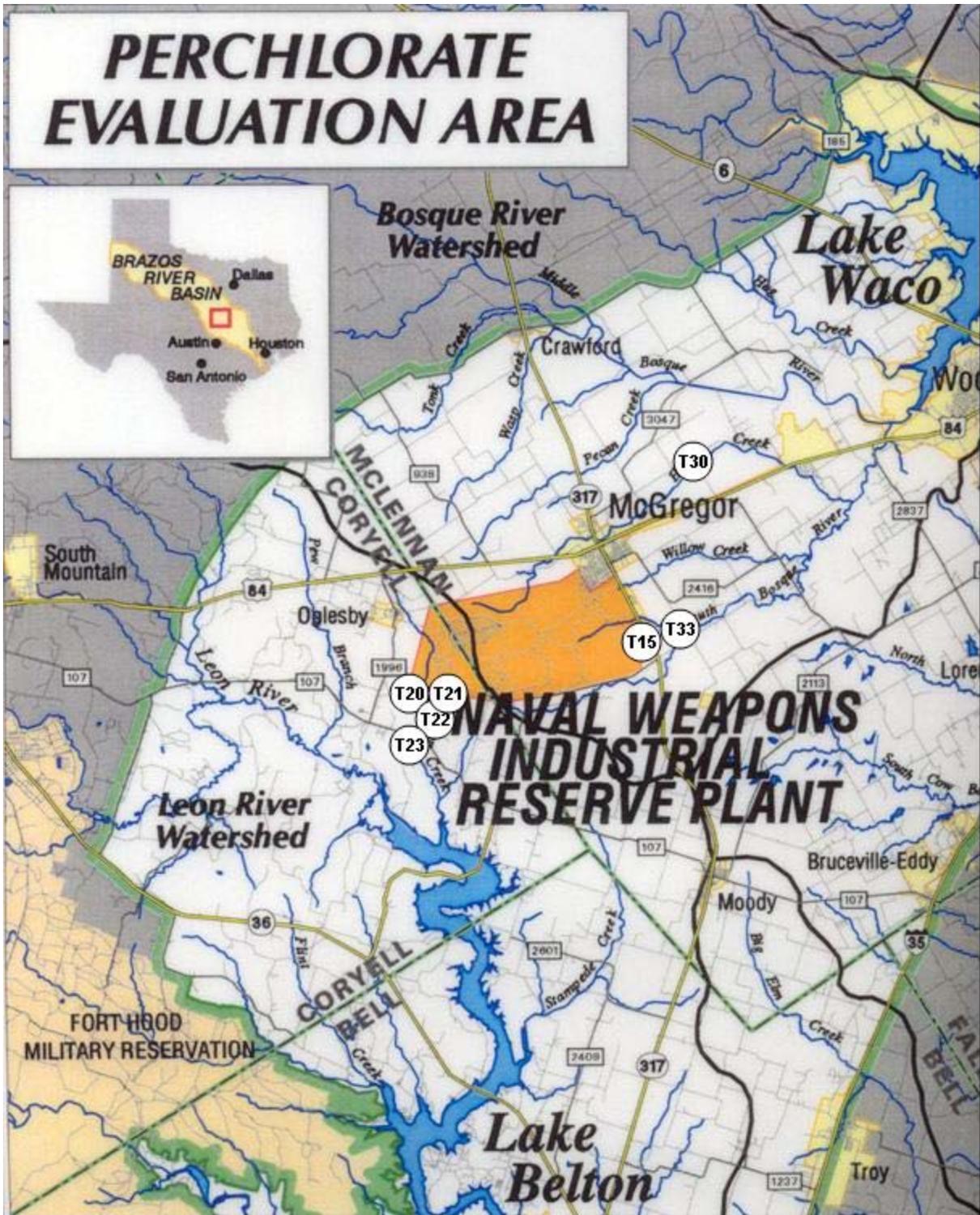


Figure 5-134
Map of Study Area Illustrating the Approximate Locations where Market Basket
Samples Were Collected

5.3.4.2.3 South Bosque River at Indian Trail (T33)

This location was also near a longitudinal stream sampling station that was part of the overall project. The homeowner at this location allowed us to collect samples from the garden. The home owner indicated that water from the South Bosque was used to irrigate the garden. Water, soil, and okra were collected from this location.

5.3.4.2.4 S Creek at Highway 317 (T15)

This location represented the most contaminated stream in the study area based on monitoring data. The only resident near this stream before it flows into the South Bosque did not have a garden, however, we collected surrogate crop samples from this area. Samples collected included water, soil, leafy vegetation, wild squash, and field corn.

5.3.4.2.5 Additional Vegetable Samples

During the course of the study, vegetable samples from other locations were collected (along with irrigation water) in order to help complete the market basket survey dataset. These samples included: (1) vegetables (cucumber, tomato, lettuce, peppers) from 4 locations in New Mexico along with the corresponding irrigation water from each location, (2) vegetables/crops (tomato, wheat, etc.) from Gaines County in West Texas and the corresponding irrigation water, (3) vegetables (cucumber, tomato, and cantaloupe) from a garden in Kansas near a slurry explosives site and irrigation water from that location, and (4) organic-grown lettuce from Northern California (not irrigated with Colorado River water, but may have been fertilized with Chilean nitrate) and the corresponding irrigation water.

5.3.4.3 Data

Results from this study are shown in **Table 5-26**. This table includes results from within the study area as well as results from other perchlorate-impacted areas.

**Table 5-26
Perchlorate Residues in Related Water, Soil, and Vegetation Samples**

(Vegetation was collected to represent food items or surrogates for food items likely consumed by humans.)

Location	Perchlorate (ppb)			Accumulation Factor
	Water	Soil	Vegetation	
Harris Creek at Windsor Road	ND	ND	Potato - ND	
Station Creek at Highway 107 (T23)	NA	NA	Berries - ND Nuts - ND	
Station Creek on A&M Property (T20-T22)				
"Spring"	3	NA	Leafy Greens - 18 ppb	≤ 6
"Water Crossing"	134	NA	Leafy Greens - 654 ppb	
"Ponded Water"	5	NA	Sunflower - 31 ppb	
South Bosque at Indian Trail (T33)	ND	ND	Okra - 2 ppb	
S Creek at Highway 317 (T15)	NA	ND	Smartweed - 1756 ppb	
			Milkweed - 3 ppb	
			Squash - 22ppb	
			Squash - 46 ppb	
			Field Corn - ND	

Location	Perchlorate (ppb)			Accumulation Factor
	Water	Soil	Vegetation	
Los Alamos County, New Mexico				
"PA"	ND	NA	Chile - 128 ppb	
			Squash - 99 ppb	
			Tomato - ND	
			Lavender - ND	
			Rhubarb - ND	
"LA"	ND	NA	Lettuce - J	
			"chanto" - 57 ppb	
			Pepper - ND	
			Lettuce - J	
			Tomato - ND	
"C"	ND	NA	Squash - ND	
			Cucumber - ND	
			Pumpkin - ND	
"SCP"	ND	NA	Chile - ND	
			Tomato - ND	
			Lettuce - ND	
			Egg Plant - ND	
			Chile - ND	
			Tomato - ND	
Gaines County, Texas	2-20 ppb	NA	Corn - ND	≤ 20
			Pepper - ND	
			Tomato - ND	
			Cucumber - 40 ppb	
Morris County, Kansas	81 ppb	NA	Cucumber - 766 ppb	≤ 20
			Cantaloupe - 1645 ppb	
			Tomato - 221 ppb	
			Tomato - 42 ppb	
Northern California	ND	NA	Lettuce - 86 ppb	
			Lettuce - ND	
			Lettuce - ND	
			Lettuce - ND	
			Lettuce - ND	

NA = no sample collected (dry stream or no relevant soil sample)

ND = not detected

See **Appendix C** for historical data

5.3.4.4 Discussion

Laboratory studies and field sampling within the study area indicate that perchlorate is readily accumulated in plants. Vegetation in perchlorate-contaminated areas or crops grown with perchlorate-contaminated water could represent a significant route of perchlorate exposure to higher organisms, including humans. However, specific conditions within the study area lessen the probability of human exposure to perchlorate through vegetation. This conclusion is based on (1) where perchlorate occurs in surface water and the location of gardens irrigated by those streams and/or well water, (2) the concentrations of perchlorate observed in most streams and the respective accumulation

factors (≤ 20), and (3) the accumulation of perchlorate in plants (tree leaves) that are not typically consumed by humans.

Although the types of food items sampled within the study area were limited (primarily by the lack of gardens located along contaminated streams), additional food items collected from other areas in the U.S. suggest that the data collected in this study are consistent. In instances where contaminated water is used to irrigate gardens, perchlorate does accumulate in plants.

5.3.5 Mathematical Modeling of Perchlorate in Plants

5.3.5.1 Introduction

Models were developed to predict perchlorate uptake in terrestrial and aquatic plants. These models provide predicted perchlorate concentrations in vegetation that can be used in subsequent models of perchlorate uptake in terrestrial and aquatic animals. The aquatic vegetation models included an algae model and an aquatic macrophyte model.

5.3.5.2 Methodology

The models are based on the CERES model, originally developed by Dixon et al. (1978). CERES utilizes a combination of the compartmental and experimental systems in its modeling approach (**Figure 5-135**). The compartmental system approach focuses on tracing the quantities of substrates in the system, while the experimental component approach utilizes detailed analysis and mathematical representation to describe environmental and physiological processes. The modeled compartments in the model are roots, stems, leaves, and fruits. Each compartment is further sub-divided into sugar substrate, storage, water, and perchlorate amount.

Both models were programmed in Matlab; the algae model was programmed using differential equations and the aquatic macrophyte model was programmed using difference equations.

For the algae model, we used the model for growth and production of algae contained in The Enhanced Stream Water Quality Model QUAL2E (Brown and Barnwell, 1987). This model was used because of extensive experience using it in a risk assessment of atrazine (Solomon et al., 1996).

The second plant model simulates the uptake, transport, and distribution of the perchlorate anion in the vegetation of various terrestrial and aquatic plant species. The intrinsic processes that are incorporated in the model include plant growth, nutrient uptake and transport, nutrient and substrate storage, plant-water relations, photosynthesis, and respiration. The important extrinsic processes or factors include solar radiation, environmental temperature, and soil hydrology.

The models were tested with each of the structural tests described in Section 5.5.1.3.2.1 and passed each test. The parallel test, comparing the output from the Matlab model with that of the FORTRAN model, showed equivalent output.

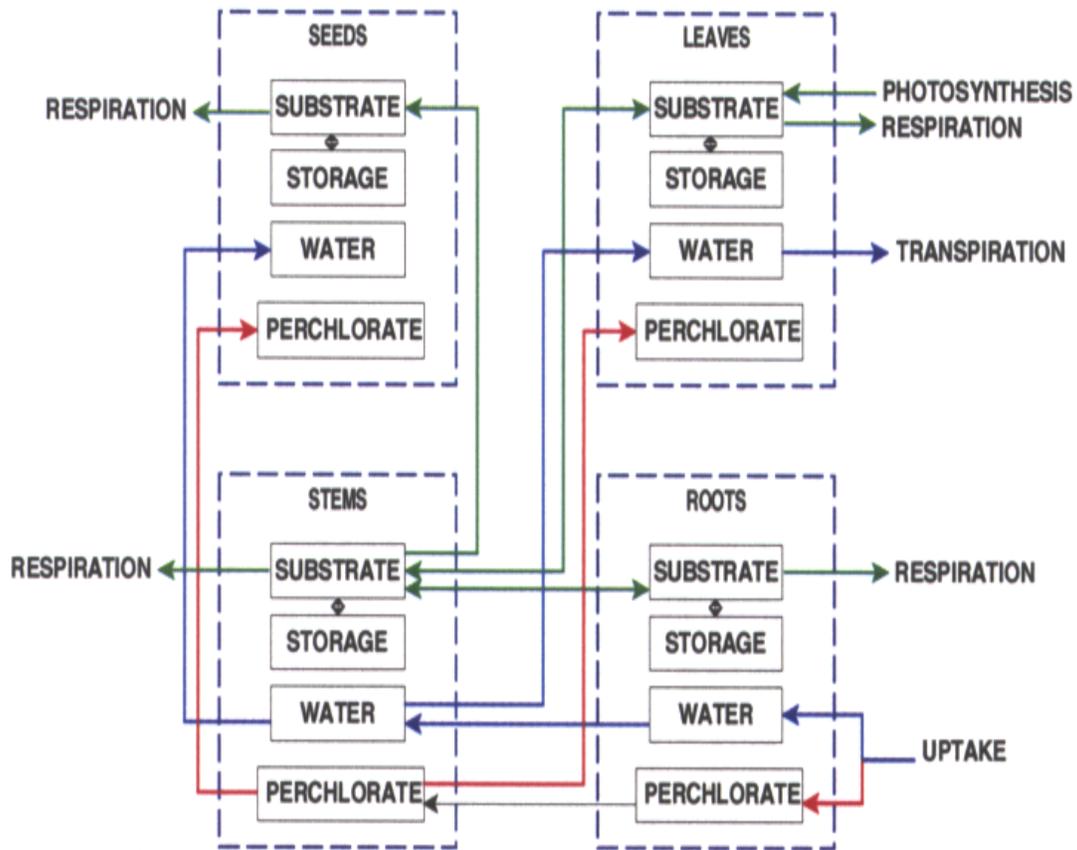


Figure 5-135
Flow Diagram of Macrophyte Model

5.3.5.2.1 Macrophyte Model Description

For the macrophyte model governing equations, we used those described by Dixon, et al. (1978). The plant's water uptake is the product of the plant's ability to take up water, its leaf area, and the volume of water available to the plant in its growing soil horizon:

$$U_t^i = f \cdot L'_{(t)} \cdot V_A$$

where :

U_t^i = incremental water uptake at time (t)

f = water flow constant

L' = Leaf Area Index

V_A = Volume of water in Soil Horizon A.

Distribution of water and perchlorate between compartments is defined by the difference in water and perchlorate between compartments:

$$F_{ab} = \begin{cases} (W_a - W_b) / r_{ab} & t_1 < t \leq t_4 \\ 0 & \text{otherwise} \end{cases}$$

where:

F = flux from compartment A to compartment B (g/m² land area/hour)

W = amount of water in a given compartment (g/m² land area)

r_{ab} = water flux constant.

The amount of perchlorate in individual compartments is defined by:

$$M_a = F_{ab} \cdot C_{\text{ClO}_4^-}$$

where:

M_a = amount of perchlorate in the compartment at each time step (t)

F_{ab} = flux of water between the two involved compartments

C_{ClO₄⁻} = ClO₄⁻ concentration in the incoming water.

The ratio of the amount of perchlorate in the compartment to the biomass of the compartment determines the perchlorate concentration:

$$Q_{a,t} = \frac{M_{a,t}}{B_{a,t} + W_{a,t}}$$

where:

Q_{a,t} = concentration of perchlorate in compartment a at time t (μg•g⁻¹)

M_{a,t} = amount of perchlorate in compartment a at time t (μg•m⁻²)

B_{a,t} = biomass of a given compartment (g•m²)

W_{a,t} = mass of water in compartment a at time t (g•m²)

Plant biomass is calculated by summing the soluble and insoluble photosynthate fractions (Dixon, et al., 1978):

$$B_{a,t} = S_{a,t} + ST_{a,t}$$

where:

B_{a,t} = biomass of compartment a at time t (g•m²)

S_{a,t} = sugar substrate in compartment a at time t (g•m²)

ST_{a,t} = plant storage tissue in compartment a at time t (g•m²)

Model Assumptions:

- transport between leaves and stems occurs from the time of bud formation to the time of abscission.

- transport between stems and fruits occurs from the time of net photosynthesis to the time of abscission.
- transport between the stems and roots is assumed to occur throughout the year.

5.3.5.2.2 Algae Model Description

We used the model for growth and production of algae contained in The Enhanced Stream Water Quality Model QUAL2E (Brown and Barnwell, 1987). The model is expressed as the differential equation:

$$\frac{dA}{dt} = \mu A - \rho A - \frac{\sigma_1}{d} A$$

where:

A	=	algal biomass concentration, $\text{mg}\cdot\text{L}^{-1}$
t	=	time, hours
μ	=	the local specific growth rate, h^{-1}
ρ	=	the local respiration rate of algae, h^{-1}
σ_1	=	the local settling rate for algae, $\text{m}\cdot\text{h}^{-1}$
d	=	average depth, m

We changed the original time step of a day to an hour. In the QUAL2E model, the algal specific growth rate is written as a function of light, and the nutrients nitrogen and phosphorus. For the purposes of these simulations, we assumed that nutrients are not limiting. Because no significant effect of perchlorate was observed, we did not include a growth limitation factor for perchlorate. In our growth experiments, we only measured net growth rate and not gross photosynthetic rate and respiration rate independently. Therefore, we used a revised model of algal dynamics in which growth rate, μ , and respiration rate, ρ , are both coupled to the expressions for the limitation factors for light, FL:

where:

$$\mu = \mu_{\max}(FL)$$

μ_{\max}	=	maximum specific algal growth rate constant, h^{-1}
FL	=	algal growth limitation factor for light

$$X_T = X_{20} \theta^{(T-20^\circ)}$$

Both μ_{\max} and μ are functions of temperature (Brown and Barnwell, 1987):

where:

X_T	=	the value of the coefficient at the local temperature (T)
X_{20}	=	the value of the coefficient at the standard temperature (20 C)

θ = an empirical constant for each reaction coefficient

Of the three options for the light functions in QUAL2E, we selected Smith's function. In this option, the algal growth limitation factor for light is formulated to include second order effects of light intensity:

$$FL_z = \frac{I_z}{\sqrt{K_L^2 + I_z^2}}$$

where:

FL = algal growth attenuation factor for light at intensity I_z
 I_z = light intensity at a given depth (z), $\text{cal}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$
 K_L = light intensity at 71% of the maximum growth rate, $\text{cal}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$
Z = depth variable, m

In QUAL2E, the light intensity, I_z , varies with depth according to Beer's law:

$$I_z = e^{-Kz}$$

where:

I = surface light intensity, $\text{cal}\cdot\text{cm}^{-2}\cdot\text{min}^{-1}$
K = light extinction coefficient, m^{-1}
Z = depth variable, m

Uptake of perchlorate from water into algal cells was assumed to follow from passive diffusion:

$$\frac{dC_{\text{cellH}_2\text{O}}}{dt} = d(C_{\text{H}_2\text{O}} - C_{\text{cellH}_2\text{O}})$$

where:

$C_{\text{cellH}_2\text{O}}$ = soluble perchlorate concentration in algal cells, ppb
 $C_{\text{H}_2\text{O}}$ = water perchlorate concentration, ppb
d = diffusion coefficient, h^{-1}

Perchlorate concentration in algae water was calculated as the concentration in algal cells multiplied by the fraction of algal biomass in water. We assumed an adsorptive mechanism between cellular water concentration and insoluble algal biomass:

$$\frac{dC_{\text{algae}}}{dt} = k_1 \cdot C_{\text{cellH}_2\text{O}} - k_2 C_{\text{algae}}$$

where:

- C_{algae} = insoluble algal perchlorate concentration, ppb
- A = algal biomass, ppm
- k_1 = adsorption rate coefficient, h^{-1}
- k_2 = dissociation/degradation coefficient, h^{-1}

Algal perchlorate concentration was calculated as both adsorbed and absorbed perchlorate divided by the algal biomass.

5.3.5.2.3 Algae Model Calibration

We used data from laboratory studies on uptake of perchlorate into duckweed (*Lemna minor*) to calibrate the model. Depth and settling rate in the lab experiments were assumed to be zero. Initial algal biomass was set to 0.55 g. We allowed the simulated algal biomass to increase over the 10 day simulation experiment (**Figure 5-136**). Water concentration was set to 100 ppb. Predicted perchlorate concentration in duckweed showed dynamics similar to observed values (**Figure 5-137**). We concluded that the model was calibrated sufficiently to conduct additional simulation experiments.

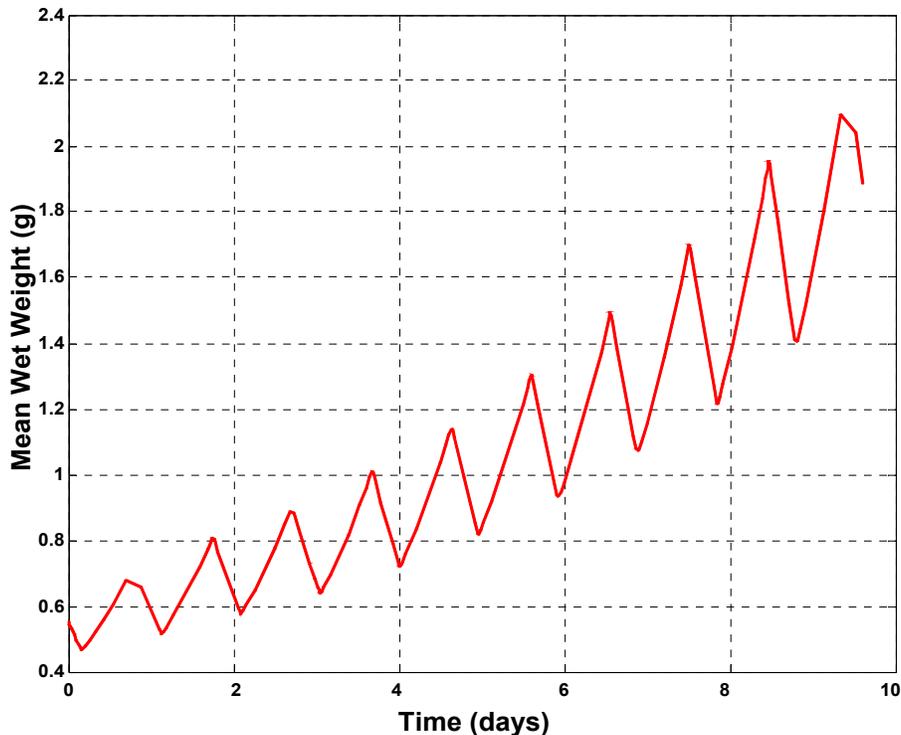


Figure 5-136
Simulated Algal Growth in Ten Day Experiment

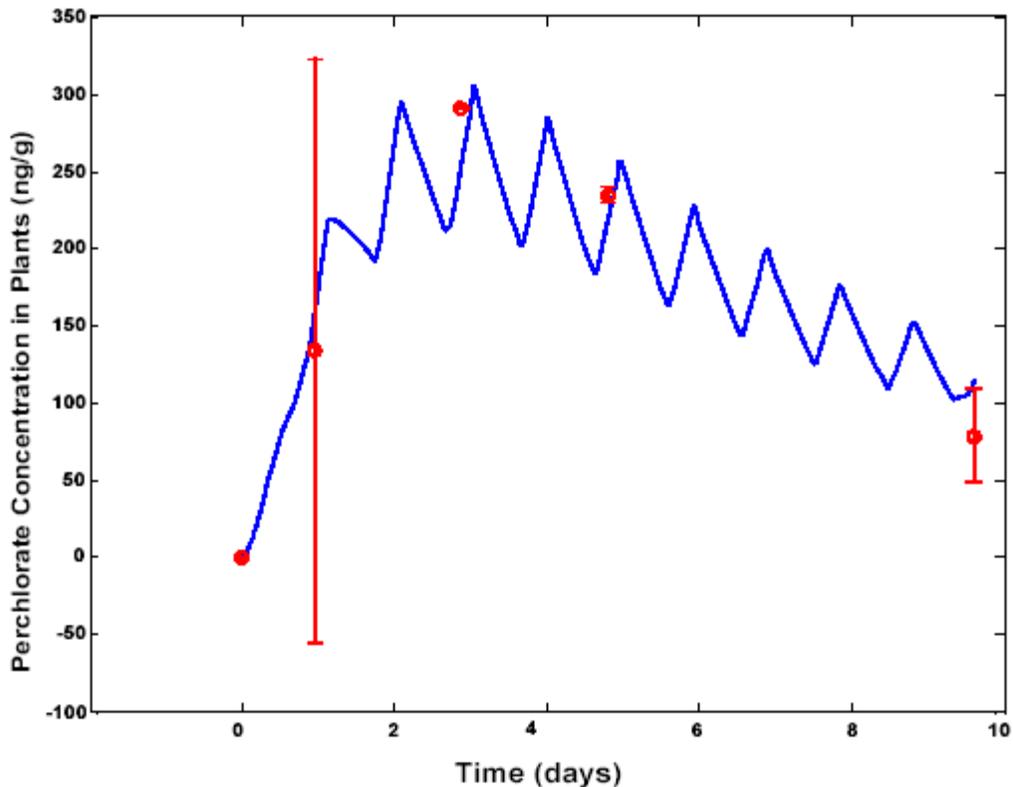


Figure 5-137
Predicted and Observed Perchlorate Concentration in Duckweed in Ten Day Experiment

(Predicted values are in blue. Observed values, in red, are mean \pm standard deviation.)

5.3.5.3 Data

5.3.5.3.1 Exposure Scenario

Simulations representing “worst-case” scenarios were run for terrestrial plants (a generic tree species), aquatic macrophytes (*Polygonum* spp), and algae. For the terrestrial plants and aquatic macrophytes, we used data collected from S Creek at Highway 317 (T15). The perchlorate concentrations used 270.48 ± 157.56 ppb. We assumed that both terrestrial and aquatic plants were exposed to the same concentrations. Simulations were run for algal populations in Harris Creek at Highway 317 (T13), the only sampling location that had a detectable perchlorate concentration (7.17 ppm) in algae.

5.3.5.3.2 Aquatic Macrophyte Simulations

The simulation results for *Polygonum* spp. (**Figure 5-138**) show an initial increase in perchlorate concentration at the start of the growing season. As plant biomass increases, relative to perchlorate uptake, there is a small decrease in concentration. When the plants reach maximum growth, biomass remains relatively constant, uptake of perchlorate continues, and concentration also increases. At the end of the growing season, both

biomass and perchlorate decrease as a result of senescence and mortality, resulting in a decrease in concentration.

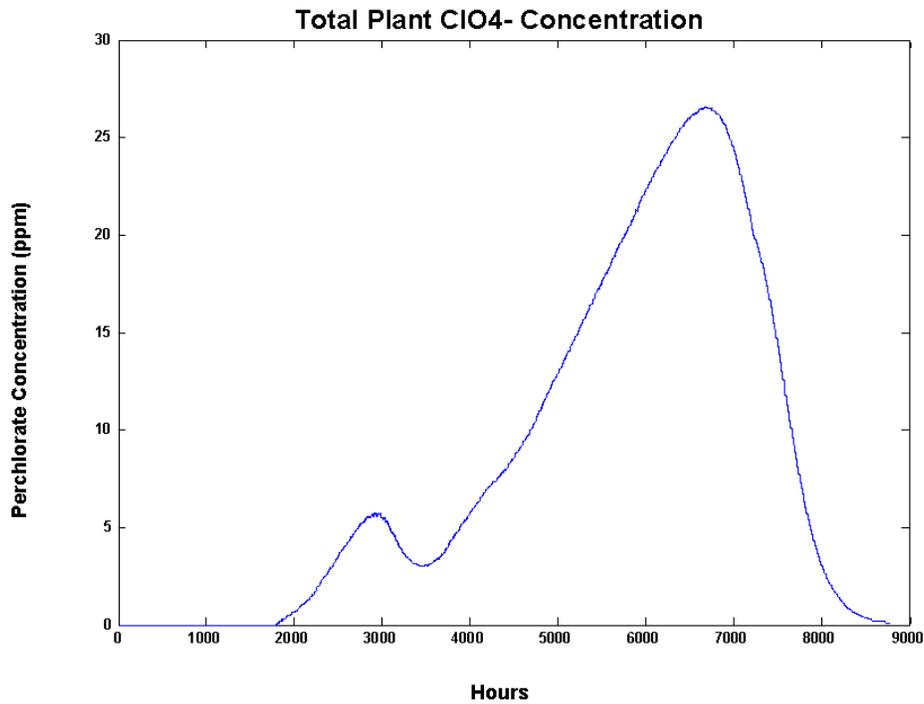


Figure 5-138
Predicted Perchlorate Concentration (ppm) in *Polygonum* spp. from S Creek at Highway 317 (T15)

The simulation results for each plant compartment show predicted perchlorate concentrations in each compartment (**Figure 5-139**).

The concentration pattern at the beginning of the growing season is similar in all compartments. There is an initial increase in concentration resulting from a relatively rapid uptake of perchlorate compared with plant growth. As plant biomass increases, there is a decrease in concentration, as reflected in the total plant concentration. There are significant differences, however, in the seasonal concentration dynamics. In the root compartment, the concentration remains at a relatively low level as the root biomass increases relative to concentration. In the stem compartment, the levels of biomass and concentration stay relatively the same, resulting in a concentration that remains at the same level. The concentrations in both the leaves and fruits increase significantly more than in the roots and stems. This is a result of the water being transpired from those compartments and the sequestration of perchlorate. The model predicts that the concentrations in both leaves and fruits continue to increase after the end of the growing season. Although there is no perchlorate accumulating in the leaves during this time of year, the amount of perchlorate decreases slightly less than the leaf biomass, resulting in a high concentration.

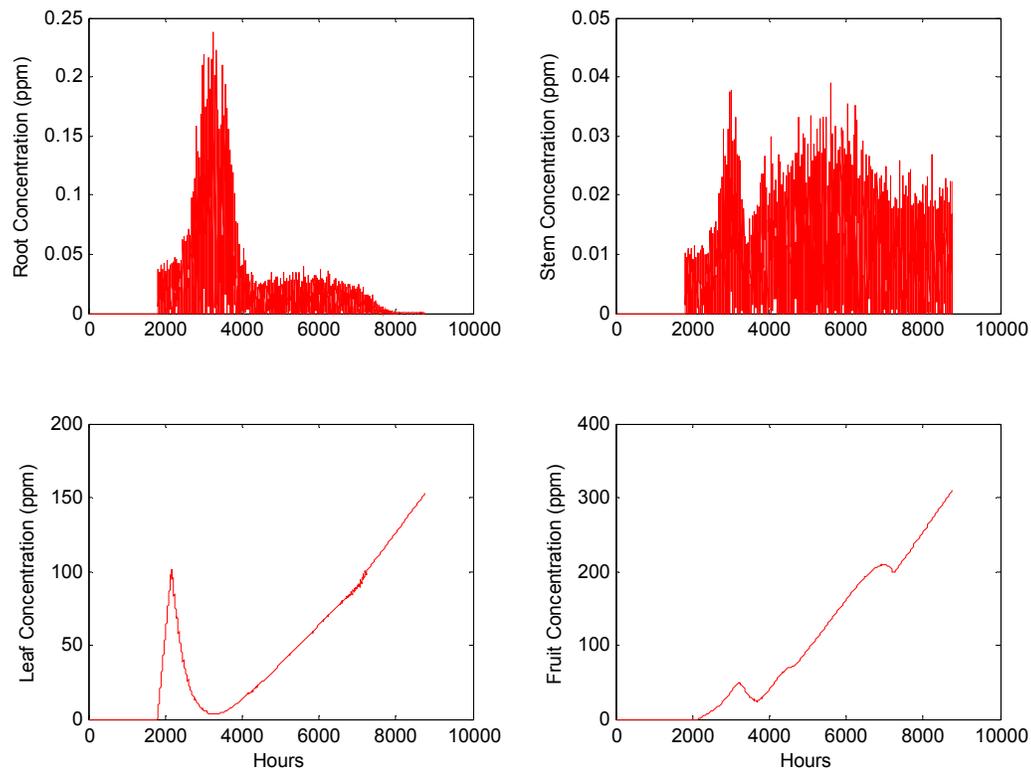


Figure 5-139
Predicted Perchlorate Concentrations in Roots, Stems, Leaves, and Fruits in
***Polygonum* spp. from S Creek at Highway 317 (T15)**

5.3.5.3.3 Terrestrial Plant Simulations

The simulation of terrestrial plants, representing perennial tree species, differed from the simulation of *Polygonum* spp. in several respects (**Figure 5-140**). The most obvious difference is that perennial plants begin with significant stem and root biomass. This tends to reduce the concentration in all compartments. Also, there is less water available in the soil compared with wetland sites. A third factor is that the height of trees increases the resistance to water movement throughout the plants. The dynamic patterns of perchlorate concentration, however, are quite similar to those of *Polygonum* spp. The predicted perchlorate concentration in tree leaves on October 29 is 15.75 ppm compared to the mean sampled concentration on that date of 21.90 ppm.

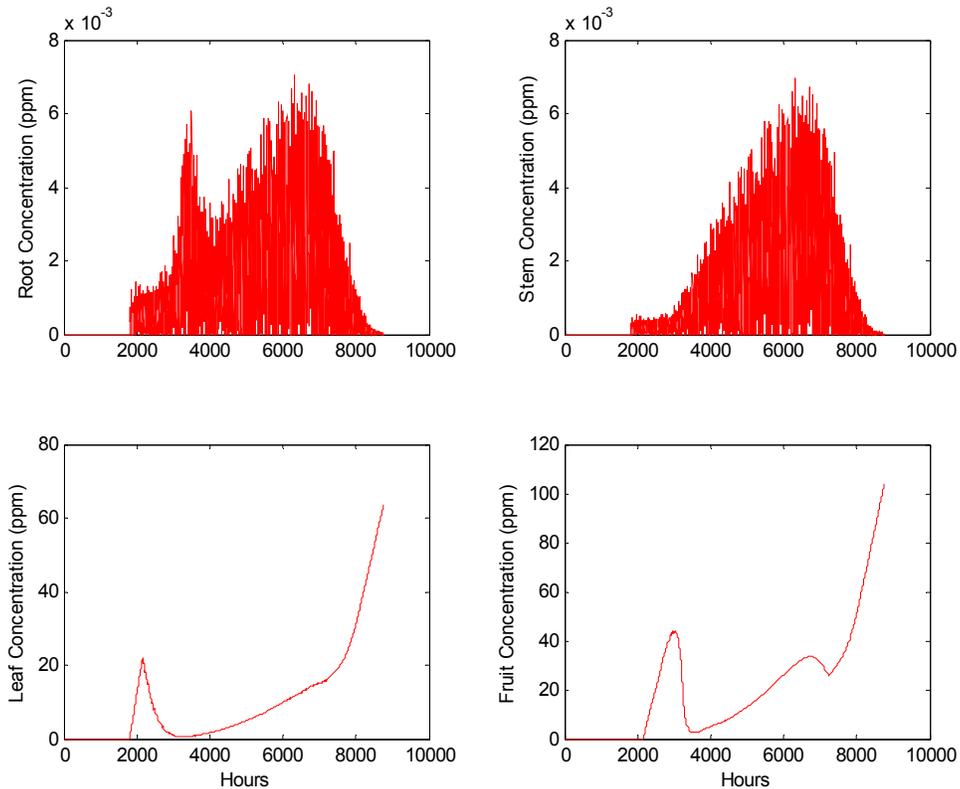


Figure 5-140
Predicted Perchlorate Concentrations in Roots, Steams, Leaves, and Fruits in Tree
leaves from S Creek at Highway 317 (T15)

5.3.5.3.4 Algae Simulations

The water concentration at the Harris Creek sampling site averaged 11.28 ppb after September 6, 2001. That value was used as the exposure concentration. These simulation results show predicted algal biomass increases seasonally (**Figure 5-141**). Perchlorate concentration increases to a peak after about 20 days into the growing season but then decreases as biomass increases (**Figure 5-142**). There is an increase in perchlorate concentration at the end of the growing season as biomass decreases but because of the low algal biomass density, the perchlorate exposure through the aquatic food chain is low. The predicted perchlorate concentration on October 6 is 4.65 ppm dry weight compared with the observed value of 7.17 ppm.

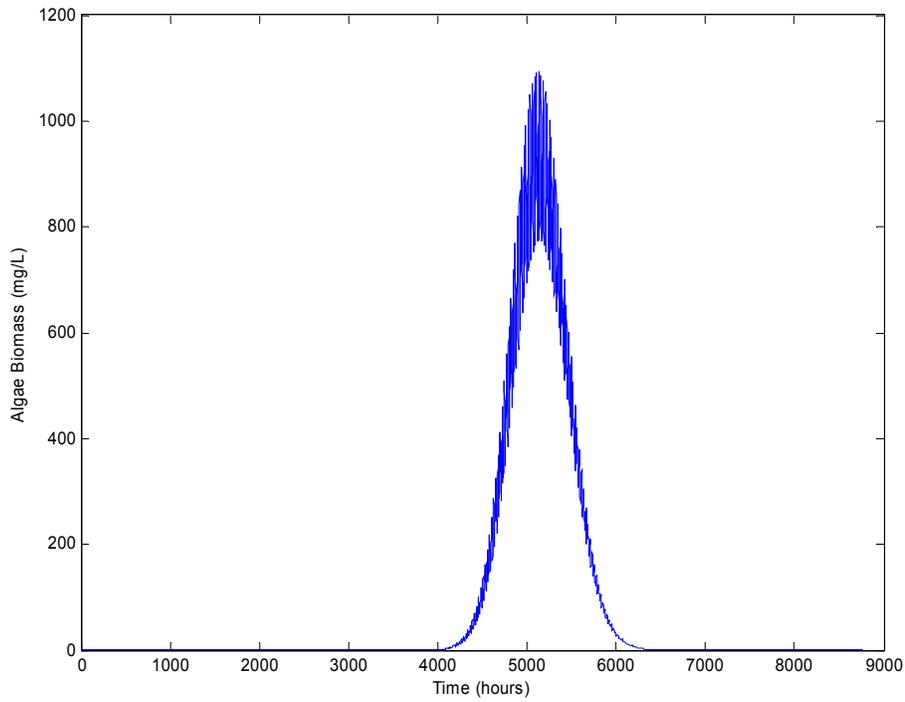


Figure 5-141
Predicted Algal Biomass in Simulated Exposure to Perchlorate Concentration from Harris Creek Site (T13)

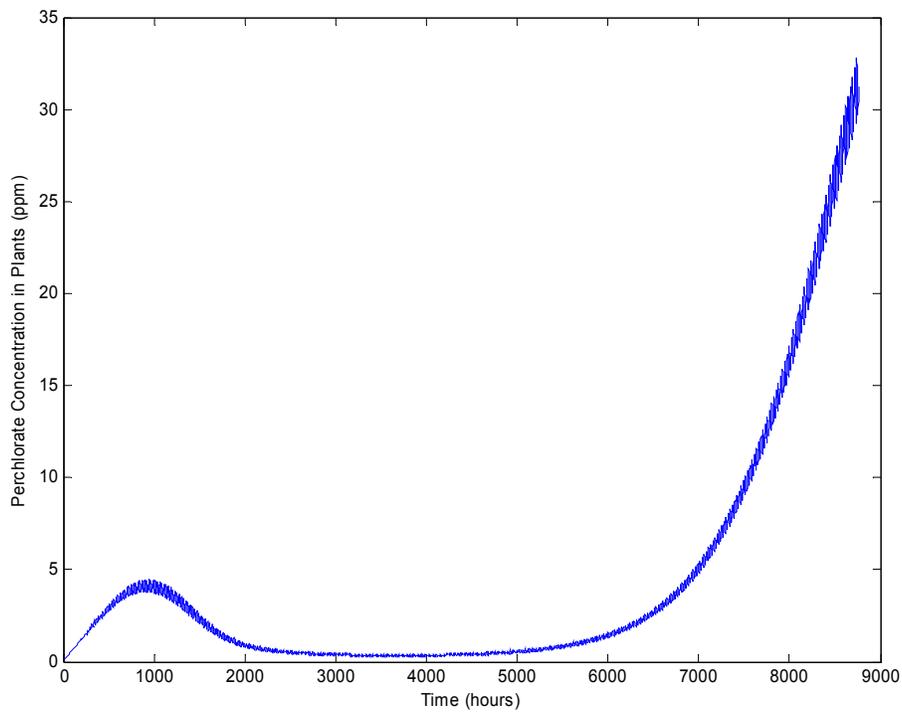


Figure 5-142
Predicted Algal Perchlorate Concentration for Harris Creek (T13) Simulation

5.3.5.4 Discussion

The vascular plant model was developed under the assumption that water is the driving force behind the uptake and distribution of perchlorate in plants. Because the model predicts tissue concentrations that are in line with laboratory and field values, it is reasonable to assume that water movement in plants is an important driving force in the uptake and distribution of perchlorate.

The model also indicates that perchlorate is capable of bioaccumulation in the leaves and fruits of exposed plants. If this result is true, there is significant potential for trophic transfer of perchlorate if wildlife and humans consume exposed plants. Although parameter estimates were based on calibration with lab experimental data, direct parameter estimation may improve the accuracy of the model predictions.