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POTENTIAL SPECIES FOR PHYTOREMEDIATION OF PERCHLORATE

by

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FOREWORD

Perchlorate has contaminated surface and groundwater resources at various locations in the United States. Concern over perchlorate contamination is due to the influence of this compound on thyroid gland function. As a potential endocrine disruptor, perchlorate can threaten the health of both human and wildlife populations.

Technologies commonly used to remove contaminants from ground and surface water either are ineffective for remediation of perchlorate or too expensive for large-scale use. Microbial transformation of perchlorate has not been accomplished for concentrations prevalent at contaminated sites. This project was the first to evaluate the ability of vascular plants to remove perchlorate from solution at field concentrations and transform this contaminant into an innocuous end product. The results of these experiments suggest that vascular plants provide a rapid, inexpensive option for remediation perchlorate-contaminated sites. Site-specific "prescriptions" will be required for on-site remediation.

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EXECUTIVE SUMMARY

Phytoremediation is the use of plants to cleanse soil and water contaminated with organic or inorganic pollutants. This promising new field of research can be used for *in situ* clean up of large volumes and expansive areas of contaminated soils or waters, including ground water. Three laboratory-scale experiments were conducted to: 1) evaluate the ability of selected terrestrial, wetland, and aquatic plants to remove perchlorate from an aqueous solution; 2) compare the performance of different age classes of one plant species; 3) evaluate the role of nutrients on perchlorate removal; 4) determine the fate of perchlorate removed from solution (*e.g.*, plant tissue distribution; accumulation vs. breakdown); 5) document external plant responses to perchlorate; and 6) predict field-scale performance of the plant species evaluated. Perchlorate concentrations of 0.2, 2.0, and 20 ppm were tested in aqueous and sand treatments for ten-day periods in each experiment.

Thirteen vascular plant species were selected for evaluation in these initial experiments. Four were trees, one was an herbaceous upland species, four were herbaceous wetland species, and four were herbaceous aquatic species. The species of trees included cabbage gum (*Eucalyptus amplifolia*), sweetgum (*Liquidambar styraciflua*), eastern cottonwood (*Populus deltoides*), and black willow (*Salix nigra*). Tarragon (*Artemesia dracuncularus sativa*) was the herbaceous upland species. The herbaceous wetland species were pickleweed (aka iodine bush, *Allenrolfea occidentalis*), blue-hyssop (*Bacopa caroliniana*), smartweed (*Polygonum punctatum*), and perennial glasswort (*Salicornia virginica*). Aquatic species were waterweed (*Elodea canadensis*), parrot-feather (*Myriophyllum aquaticum*), fragrant white water-lily (*Nymphaea odorata*), and duckmeat (*Spirodela polyrhiza*).

A preliminary sorption experiment with unwashed sand and no plants revealed that 50-64% of perchlorate in solution became adsorbed to the sand, displacing chloride. Consequently, for treatments with unwashed sand and plants in the subsequent three experiments, the free chloride ions in solution were available to be taken up by the plants. When perchlorate concentrations exceeded 2.0 ppm in unwashed sand treatments, an option was available for plants to take up excess perchlorate, rather than chloride ions, from the solution.

Perchlorate was depleted from solution in the presence of all but two species (waterweed and duckmeat). The mass of perchlorate depleted (g/kg wet weight) was classified into five general categories (0 = no depletion; 1-99 = minimal depletion; 100-499 = moderate depletion; 500-999 = moderately high depletion; and >1000 = high depletion). None of the tree species tested, nor the herbaceous upland species tested were included in the highest category of performance. Wetland and aquatic plants included in the highest category were blue-hyssop, perennial glasswort, and parrot-feather. Results in the moderately-high category were obtained for one species of tree (cabbage gum), the herbaceous upland species (tarragon), and three of the four herbaceous wetland species evaluated (pickleweed, smartweed, and glasswort).

Depletion was calculated as a first-order kinetics reaction, with k values (day-1) in sand treatments in the range of 0-0.22 for cabbage gum, sweetgum, rooted green-wood cuttings of cottonwood, willow, pickleweed, smartweed, glasswort, and water-lily. Upper values for rooted mature-wood cuttings of cottonwood, blue-hyssop, and parrot-feather were 0.31, 0.34, and 0.41, respectively. The range for tarragon was 0.48-0.77. Plant tissues (*e.g.*, roots, stems, leaves) were analyzed from selected samples, based on maximum drop in perchlorate concentration, for each of the 11 species for which perchlorate depletion was observed. Perchlorate, or transformation

metabolites (chlorate, chlorite, chloride) were observed in all tissues analyzed. Future work should include: 1) a quantitative analysis of different plant tissues (*e.g.*, roots, stems, leaves), and 2) radiolabeled chloride to determine the amount and source of chloride contained within the plants.

Results suggested that significant influences on depletion of perchlorate include: 1) plant species present, 2) concentration of perchlorate, 3) substrate (sand versus aqueous treatments), 4) the presence or absence of nutrients, 5) stage of plant maturity, and 6) the presence of chloride ions. Characteristics of cottonwood and willow cuttings obtained from a site with perchlorate in the ground water, and incorporated into these experiments suggested that fungal pathogens may be present in the donor plants on the site. Fungal pathogens, if present, may have influenced the performance of these plants in the experiment. Conversely, exposure of plants to perchlorate may create stresses that result in predispostion of the plant to infection by plant pathogens. Evaluation of these factors was not within the scope of these initial experiments, but should be addressed in future experiments. Another important aspect not evaluated in these short-term experiments was the potential environmental hazard to wildlife that may consume plants used for phytoremediation, that contain high concentrations of perchlorate. Future experiments of longer duration should provide more information regarding the degree to which perchlorate is accumulated in plant tissue, and any potential threat to wildlife. The experimental design of the final two experiments, and the short duration of these experiments suggest that external microbes and algal contaminants were not involved in the depletion of perchlorate observed in these experiments.

Based on the results of these experiments and ecological knowledge of the species evaluated, the following species are recommended for initiating future research for phytoremediation of perchlorate, and are grouped by the type of phytoremediation for which they appear to be suited. Additional research using sweetgum, eastern cottonwood, and black willow is recommended for *in situ* phytoremediation of contaminated soils in uplands, including areas with shallow ground water accessible to plant roots, and if production of biomass for harvest is of interest. For *in situ* phytoremediation of contaminated areas that are saturated or inundated periodically, or for wetlands created for phytoremediation, additional research using blue-hyssop, smartweed, and perennial glasswort is recommended. Additional research using parrot-feather and fragrant white water-lily is recommended for *in situ* phytoremediation of contaminated water bodies, or for ponds created artificially for phytoremediation of contaminated surface water or extracted ground water. Finally, extracts from tarragon may be useful for injection into mechanized flow-through systems where ground water is extracted, exposed to these compounds, then reinjected into the aquifer, or for similar flow-through systems for contaminated surface water.

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1.0 INTRODUCTION

1.1 Background and Health Implications

Perchlorate, chlorate, chlorine dioxide and hypochlorite are produced on a large scale by the chemical industry, for a wide range of applications. Ammonium perchlorate is an oxyanion that has been used extensively as a strong oxidizing agent in solid rocket fuel (Ataway and Smith, 1993). Perchlorate must be removed from inventory periodically and replaced with a fresh supply, because of its shelf life. Contamination of ground water has occurred as the result of incidental discharges of perchlorate fuel used in rockets and activities associated with World War II, Korea and Viet Nam. The problem was compounded by liberal disposal practices during the 1950s through the 1970s, prior to expanded knowledge of the impacts of these fuels on soils and water resources (California Department of Health, 1998).

Wastewater generated from the manufacturing, maintenance, and testing of solid rocket propellants can contain ammonium perchlorate concentrations in the range of grams per liter. (Herman and Frankenberger, 1998). Ammonium perchlorate also is used in the production of explosives, pyrotechnics and blasting formulations. Other perchlorate formulations are used in dry batteries and oxygen-generating systems (Malmquist *et al*., 1991). The large solid rocket motor disposal inventory currently has 55 million pounds of propellant ready for treatment. Over the next 8-10 years this amount is expected to increase to 164 million pounds of solid propellant targeted for disposal (Phillips Laboratory, 1997). Innovations in fuel handling methods have reduced current contamination; however, the United States Air Force is attempting to clean-up past spills to attain environmental quality goals in balance with national defense needs.

Perchlorate currently is not regulated under the safe drinking water act, although the California Department of Health Services has established an action level for perchlorate in drinking water of 18 micrograms per liter (California Department of Health, 1998). The primary human health concern related to perchlorate is that chronic or longterm exposure can interfere with the thyroid gland's ability to utilize iodine to produce thyroid hormones required for normal body metabolism, as well as growth and development (Stanbury and Wyngaarden, 1952). Information on the health effects and toxicology of perchlorate is limited. The majority of data available regarding impacts of perchlorate on humans is from clinical reports of patients treated with potassium perchlorate for hyperthyroidism resulting from an autoimmune condition known as graves' disease (AWWARF, 1998). Potassium perchlorate continues to be used diagnostically to test thyroid hormone production. The effect of perchlorate on thyroid hormone function is the competitive inhibition of iodide anion uptake into the thyroid gland by perchlorate anion, resulting in reduced thyroid hormone production. Iodine deficiencies in pregnant women are detrimental to fetal development. Interference with the normal function of the thyroid gland suggests that perchlorate is an endocrine disruptor (van Wijk and Hutchinson, 1995).

1.2 Remediation Approaches

A number of physical and chemical processes to treat perchlorate-contaminated sites are under consideration. Some of the processes have been tested with limited success. The chlorine in perchlorate is at an oxidation state of plus seven, which is much higher than the minus one oxidation state of chloride, the most stable form of chlorine in water. This would seem to favor processes based on chemical reduction. However, pechlorate has found to be resistant to chemical reduction

processes. The factor that contributes to the chemical stability of dissolved perchlorate are the four oxygen atoms surrounding each chlorine. This results in a completely filled outer electron shell and tetrahedral packing of the four oxygen atoms. Consequently the charge is distributed evenly around a relatively large surface area. Therefore, a large activation energy is required to disrupt the stable structure of perchlorate to allow the very thermodynamically favorable reduction reaction to proceed. Because of the stability of perchlorate under most environmental conditions, reduction processes have failed in remediating perchlorate-contaminated waters (van Ginkle *et al*., 1995).

Perchlorate salts dissociate in water to form perchlorate anions and are highly soluble (>200 g/L). Therefore, volatilization technologies such as air-stripping are ineffective. Activated carbon, another common technology used for potable and wastewater treatment, also is ineffective in treating perchlorate-contaminated waters (AWWARF, 1998). Ion-exchange technology has potential for remediation, but is not used widely due to its high cost (Glass, 1998). Other advanced processes for the removal of perchlorate, such as reverse osmosis and nanofiltration technologies, also are expensive.

A biological process developed at Tyndall Air Force Base (AFB) consists of a two-step process, an anaerobic reactor followed by an aerobic reactor (Wallace *et al*., 1998). Development of this process has progressed from laboratory scale, to bench scale, to a pilot scale facility. In this microbial system, the perchlorate is reduced to chloride ions and oxygen. This process has been applied successfully to input water with a 9000 ppm perchlorate concentration, reducing the perchlorate to below 500 ppb (van Ginkle *et al*., 1996 and Rikken *et al*., 1996). However, the system has not been evaluated at lower concentrations that are prevalent at contaminated sites. Additionally, the current understanding of microbial reduction of perchlorate by pure cultures is limited because the sequential reduction of perchlorate to chlorate, chlorite, and ultimately chloride and oxygen, has not been studied in detail. In summary, no proven technology is available for the treatment of large volumes of water or soil containing relatively low concentrations of perchlorate. Consequently, the development of efficient and cost effective strategies for the remediation of perchlorate contaminated sites is of immense interest.

Phytoremediation, using plants to cleanse soil and water contaminated with organic or inorganic pollutants, is an alternative strategy. Plant-based systems provide an attractive remediation strategy because complete transformation of the compound to the end products (chloride ion and oxygen) occurs. This developmental process could be used for on-site and *in situ* clean-up of large volumes and expansive areas of contaminated soils or waters, including ground water. Initial experiments reported herein have been performed to elucidate the environmental behavior and fate of perchlorate using selected vascular plants that may have potential for remediating sites contaminated with perchlorate.

2.0 OBJECTIVES

Laboratory-scale experiments were conducted to: 1) evaluate the ability of selected upland, wetland, and aquatic plants to remove perchlorate from solution; 2) compare the performance of different age classes of single plant species; 3) evaluate the role of nutrients and chloride on perchlorate removal; 4) determine the fate of perchlorate removed from solution (*e.g.*, distribution throughout the plant; accumulation vs. breakdown); 5) document external plant responses during the experiments; and 6) predict field-scale performance of the plant species evaluated.

3.0 EXPERIMENTAL METHODS

3.1 Selection Criteria for Plant Species Evaluated

A total of 13 plant species were selected for evaluation in the initial, laboratory-scale experiments. Four of the species were trees, one was a herbaceous upland species, four were herbaceous wetland species, and four were aquatic species (Table 1). The species of trees included cabbage gum (*Eucalyptus amplifolia*), sweetgum (*Liquidambar styraciflua*), eastern cottonwood (*Populus deltoides*), and black willow (*Salix nigra*). The herbaceous upland species selected was tarragon (*Artemesia dracuncularus sativa*). The four herbaceous wetland species included pickleweed (aka iodine bush, *Allenrolfea occidentalis*), blue-hyssop (*Bacopa caroliniana*), smartweed (*Polygonum punctatum*), and perennial glasswort (*Salicornia virginica*). Four aquatic species also were selected, and included waterweed (*Elodea canadensis*), parrot-feather (*Myriophyllum aquaticum*), fragrant white water-lily (*Nymphaea odorata*), and duckmeat (*Spirodela polyrhiza*).

The primary determinant in selection of plant species for these initial experiments was plants of specific interest to the United States Air Force. Specifically, these included the four tree species listed above, tarragon, and pickleweed. Two additional tree species, sycamore (Platanus occidentalis) and hackberry (Celtis laevigata) also were of interest to the Air Force for inclusion in these initial experiments. Unfortunately, commercially-grown seedlings of these species could not be located during the time period that these experiments were being conducted and these species reportedly do not root readily from cuttings. Seedlings of sycamore, sweetgum, and possibly hackberry can be obtained from the Texas Forest Service for approximately $20/100, but may require a request a year in advance for collection of seed. Orders are placed in early October, and seedlings that are ordered are shipped, bare root, in January or February of the following year (Larry Schaapveld, Texas Forest Service, pers. comm., 6/18/98). The germination rate for sycamore reportedly is approximately 10%, consequently few growers carry sycamore (Helen Matthews, Rennerwood Nursery, pers. comm., 7/98).

The tree species referenced above were of interest to the Air Force for several reasons. First, trees generally have the ability to develop extensive root systems that are capable of colonizing large expanses of soil in areas where the water table may be contaminated with compounds such as perchlorate. Second, the species of trees selected are relatively rapid-growing trees, which may have higher rates of transpiration than trees that grow more slowly. If more water is transpired, theoretically more ground water containing a given pollutant, such as perchlorate, can be removed from the system and treated. If trees planted for phytoremediation grow rapidly, they provide a potential for commercial biomass production, which means that they can be harvested and sold as wood, pulp, or fuel products to help defray the cost of site-decontamination. Finally, the referenced species are thought to be tolerant of a wide range of growing conditions, and might be adaptable to contaminated sites at various locations throughout the United States.

Tarragon, one of the two herbaceous plants selected by the Air Force, was chosen based on the ability of enzymes released from its crushed leaves to transform perchlorate (Nzengung, unpub. data). Pickleweed, the other herbaceous species selected by the Air Force, is a succulent, high elevation desert plant that occurs in coastal areas and playas (old land-locked lake beds) throughout the Great Basin, from the southwestern U. S. (*e.g.*, Nevada, New Mexico, Texas, Wilcox Playa in Arizona, the Chihuahua Desert) to Mexico (*e.g.*, Baja California, Sonora). This species may be the most salt-tolerant plant in the country (Phil Jenkins, pers. comm. 6/11/98), and is capable of tolerating large concentrations of salts (generally chlorides) in soils saturated with brackish or saline water. Plants with these characteristics are known as halophytes. Halophytes may grow in areas of high salt content

Table 1. Plant Species Evaluated in Initial Perchlorate/Nutrient Experiments

Scientific Names	Common Names*
Trees	
Eucalyptus amplifolia Naud.	cabbage gum (3)
Liquidambar styraciflua L.	sweetgum (3)
Populus deltoides Bartr.ex Marsh.	eastern cottonwood (3)
seedlings	
mature-wood cuttings **	
green-wood cuttings **	
Salix nigra L.	black willow (2)
Upland Herbs	
Artemisia dracunculus var. *sativa* L.	tarragon (1)
Wetland Herbs	
Allenrolfea occidentalis (Watson) Kuntze.	pickleweed (2)
Bacopa caroliniana (Walt.) Robins.	blue-hyssop (3)
Polygonum punctatum Ell.	smartweed (3)
Salicornia virginica L.	perennial glasswort (3)
Aquatic Herbs	
Elodea canadensis Rich. in Michx.	waterweed (1)
Myriophyllum aquaticum (Vell.) Verdc.	parrot-feather (1)
Nymphaea odorata Ait.	fragrant white water-lily (3)
Spirodela polyrhiza (L.) Schleid.	duckmeat (2)

* number in parenthesis designates experiment to which plant was assigned
** cuttings from Carswell Air Force Base, rooted in the laboratory

by one of several means. They may have evolved mechanisms to exclude salts from entering through their roots, or to extrude salts through their leaves or other organs. Some have developed a specialized physiology to tolerate high salt content in their tissue. The latter mechanism of tolerance is the process used by pickleweed. Unlike some halophytes, pickleweed requires high concentrations of ions such as chloride to maintain its osmotic balance. This characteristic, and the presence of chloride with perchlorate formed the basis for selection of this species.

Perennial glasswort is in a closely related genus of the same family (Chenopodiaceae) as pickleweed. Perennial glasswort is a rhizomatous perennial, occurring in salt and brackish marshes and flats along the Atlantic coast from New Hampshire to south Florida, westward along the Gulf coast from Florida to Texas, from California to British Columbia, the West Indies, western Europe, and north Africa (Godfrey and Wooten, 1981). It was selected for evaluation by the researchers because it has the same physiological traits as pickleweed. It also has an extensive geographic range, appears to grow more rapidly than pickleweed, and is more tolerant of transplanting and vegetative propagation.

The remaining two herbaceous wetland species, blue-hyssop and smartweed, were selected by the researchers because of their widespread accessibility, ease of vegetative propagation, and apparent robustness. Blue-hyssop occurs naturally in wetlands and open water systems throughout the Coastal Plain, southeast from Virginia to south Florida, and westward to east Texas (Godfrey and Wooten, 1981). The species of smartweed selected by the researchers for this experiment is a vigorous perennial, also occurring in wetlands and open water systems, but with a more extensive range than blue-hyssop. Our test species of smartweed occurs throughout most of temperate and subtropical North America, and tropical South America (Godfrey and Wooten, 1981).

Four aquatic species with different growth characteristics were selected by the researchers to test the ability of aquatic plants to remove or transform perchlorate in the water column. The selected species of aquatics all have potentially broad geographic ranges, are easily propagated, and are relatively fast growing. Waterweed has dense leaves that can extend throughout the water column and across the surface of the water. It has few roots, and does not need to be rooted in a substrate. Parrot-feather has both submersed and emergent leaves, few roots, and also does not need to be rooted in a substrate. Fragrant white water-lily has large floating leaves that extend from thick rhizomes (horizontal stems) that creep along the substrate. Numerous large, spongy roots grow from the rhizome to anchor the plant to the substrate. The hardiness, large leaves and rhizomes, and the ease of reproducing this species contributed to its selection. The final species, duckmeat, was selected because it is a floating-leaved aquatic that reproduces relatively rapidly, is easily transported, and is thought to be adaptable to a wide geographic area. The working hypothesis for this species was that enzymes released from dying or decomposing plants might transform perchlorate.

Taxonomic, range and habitat information can be obtained for cabbage gum from Mabberley (1997), for the remaining tree species from Godfrey (1988), for tarragon from Bailey and Bailey (1976), for pickleweed from Jaeger (1947), and for the remaining species from Godfrey and Wooten (1979 and 1981). A description of the sources of the plants, transport, and acclimation procedures is provided in Appendix A.

3.2 Sorption Experiment

Sand was selected as the solid substrate for the perchlorate experiments. A small-scale sorption experiment was conducted prior to initiation of the perchlorate/nutrient experiments to determine whether chloride ions were associated with the sand. If chloride ions were present, these ions could be displaced into the solution in the presence of perchlorate, as the perchlorate adsorbed to the sand grains.

A series of test tubes containing 5 g of unwashed sand (All Purpose, Setcrete, Inc.) per test tube was equilibrated with10 mL of sodium chloride solution at one of five test concentrations (0, 50, 100, 200, 400 mg/L) for 24 h. The test tubes were shaken on a rotary shaker overnight at room temperature. After saturation with sodium chloride solution, the supernatant solution was replaced with 10 mL of 200 ppm perchlorate solution and shaken overnight. The following day, 1 mL of solution was filtered and analyzed for chloride and perchlorate concentrations using Dionex Ion-chromatography. The experiment was duplicated.

3.3 Kinetics Experiments

Laboratory-scale experiments were conducted for an initial determination of the kinetics of perchlorate depletion from solution in the presence of selected species of trees, upland, wetland, and aquatic herbs. Three concentrations of perchlorate, differing by an order of magnitude each (0.2, 2.0, and 20.0 ppm), were selected for evaluation (Figure 1). The highest concentration of perchlorate evaluated was selected because it is equivalent to field concentrations of groundwater contamination at sites of interest. The first run of these experiments contained three treatments with three experimental units per treatment and one concentration of perchlorate per unit, as shown in Figure 1. Run 1 also included two control treatments (Figure 1). Each experimental unit consisted of a 600-mL beaker and an experimental plant (for duckmeat, 3 g wet weight of plants were used), in addition to the other assigned treatment components. The experimental design for Run 1, shown in Figure 1, was used to evaluate tarragon, waterweed, and parrot-feather. All purpose sand (Setcrete, Inc.) was used in all treatments except the "no-sand" treatments. The influence of nutrients on perchlorate depletion was evaluated by adding a dilute solution (0.1 g/L) of Peters Professional All Purpose Plant Food to some of the treatments, with the paired treatments containing an equivalent volume of deionized (DI) water. The contents of the nutrient source are provided in Appendix B.

For treatments containing sand, approximately 320 g of sand were weighed into each beaker and 300 mL of perchlorate solution at a known concentration (0.2-20.0 ppm) was added. Perchlorate solutions were made either in Peters solution or in DI water prior to the run. Comparable specimens of the three experimental species of plants for Run 1 were selected and numbered sequentially for each species. Each plant then was assigned randomly to one of the treatments. The bare roots were rinsed in DI water to free any remaining soil, and the roots were pressed lightly between paper toweling to remove excess water. The fresh weight of each plant was determined and recorded prior to placement of the plant in the beaker. For treatments with sand, the assigned plant was placed in the beaker in such a manner that the surface of the sand was at the top of the root zone. For treatments without sand, the assigned plant was maintained in an erect position by placing four strips of tape across the top of the beaker in a cross-hatch pattern on four sides of the stem.

The top of each beaker was covered loosely with a plastic wrap to minimize loss of solution due to evaporation. The experimental units were placed under Sylvania spot gro lights (150w, 120v), that provided a full photosynthetic spectrum at 400 to 500 E m-2 s-1 at 20-30 cm above the plant. Lights remained on continuously for all experiments. The experiments were conducted at ambient laboratory temperature (20o C). The pH of the no-sand and washed-sand treatments was 7.0, while for unwashed-sand it was at 8.5.

Based on the results of the sorption experiment and responses of some of the plants tested in the initial perchlorate/nutrient experiments, some of the plant responses appeared to be due to chloride toxicity. Consequently, the original experimental design was refined for the subsequent two experiments to resolve these problems, and to incorporate other improvements. The modified designs and plant species used for the two subsequent experiments are provided in Figures 2 and 3.

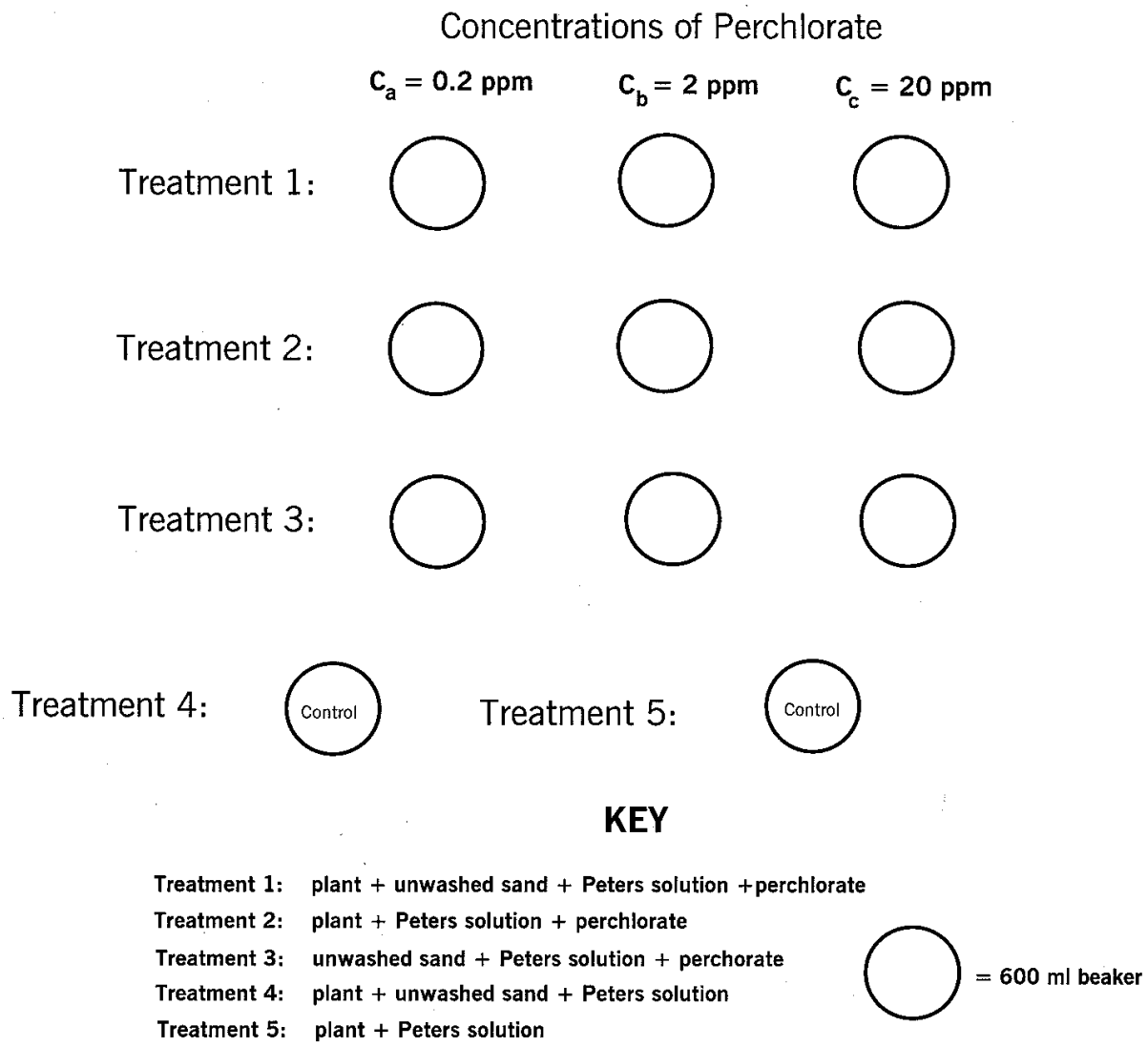

Figure 1. First Set of Initial, Ten-Day Perchlorate/Nutrient Kinetics Experiments Using Tarragon (n=11), Waterweed (n=11) and Parrot-feather (n=ll).

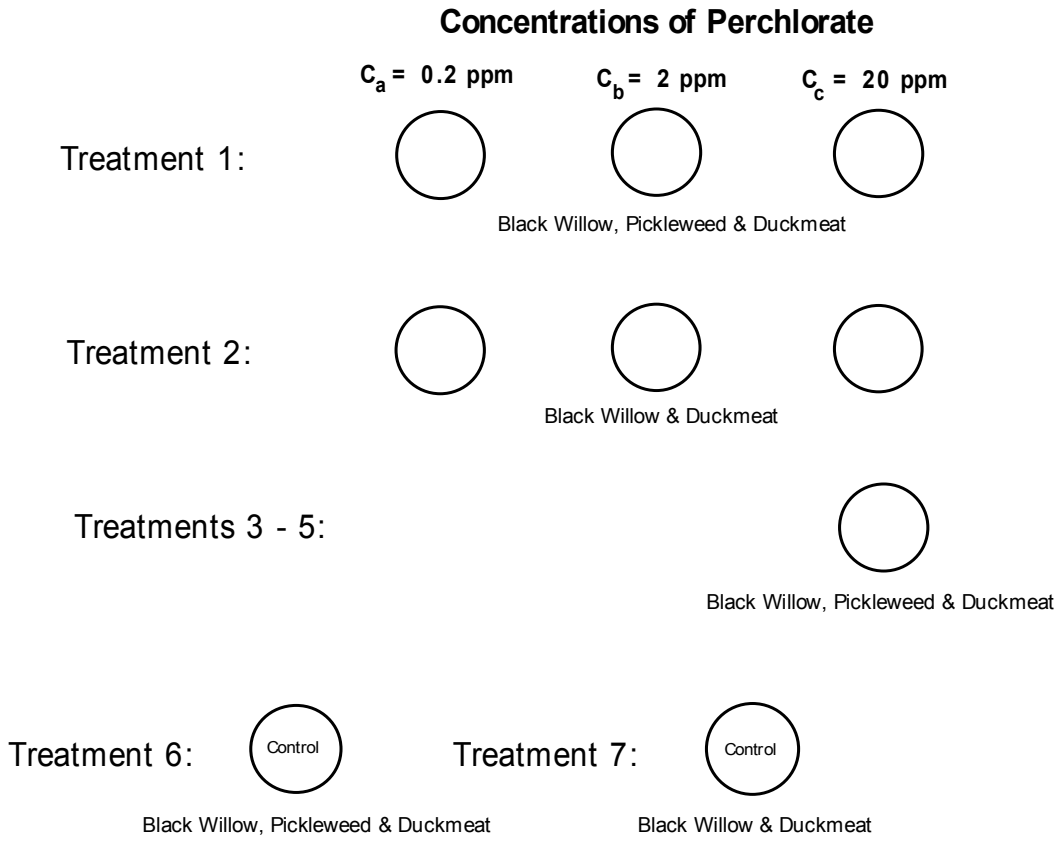

Figure 2. Second Set of Initial, Ten-Day Perchlorate/Nutrient Kinetics Experiments Using Black Willow (n=11), Pickleweed (n=7) and Duckmeat (n=11).

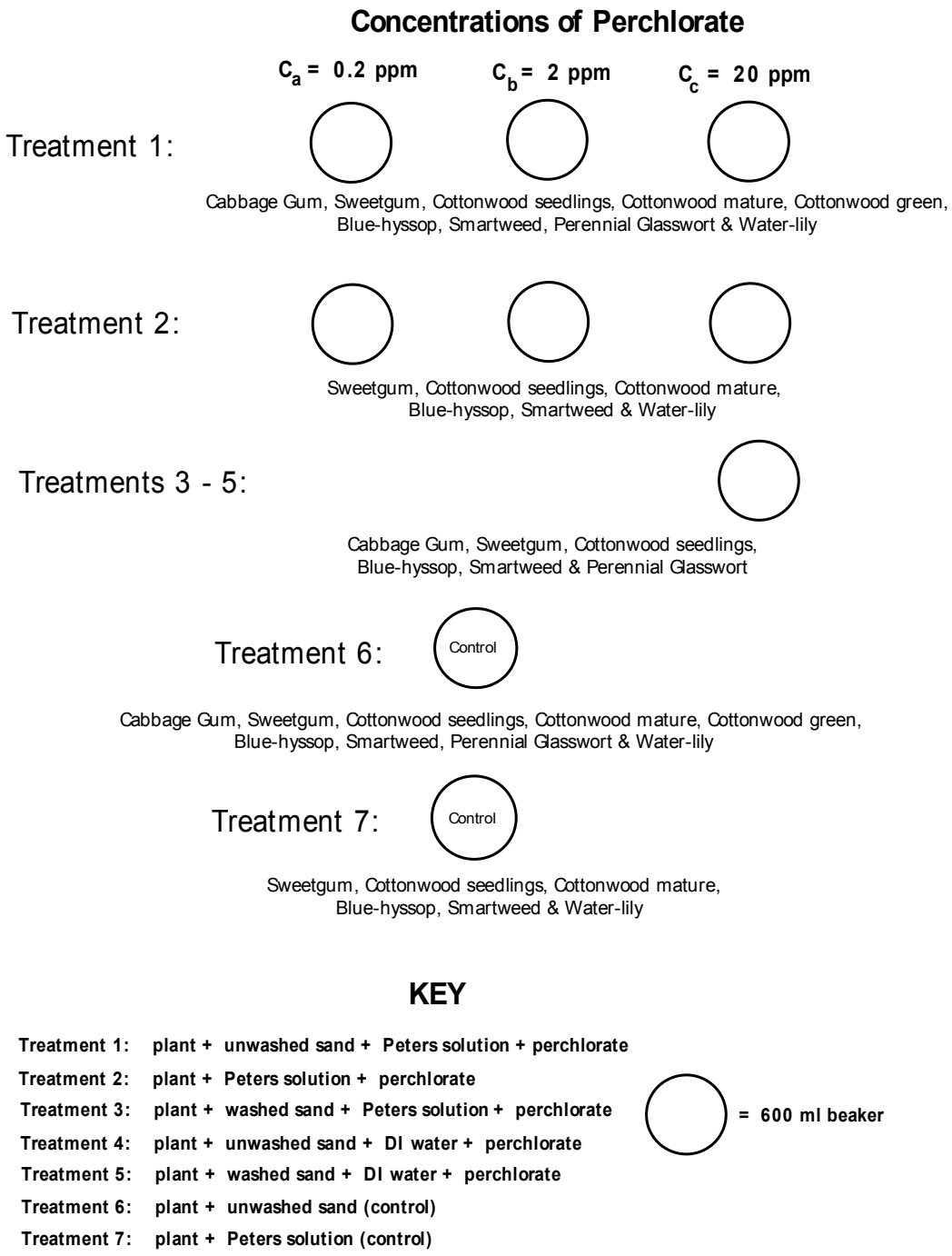

Figure 3. Third Set of Initial, Ten-Day Perchlorate/Nutrient Kinetics Experiments Using Cabbage Gum (n=7), Sweetgum (n=ll), Eastern Cottonwood (Seedlings (n=11) Mature-wood Cuttings (n=8), and Green-wood Cuttings (n=4)), Blue-hyssop (n=11), Smartweed (n=11), Perennial Glasswort (n=7), and Fragrant White Water-lily (n=8).

In experiments 2 and 3, the sand to be used in Treatments 3 and 5 was washed to remove free chloride. The initial wash was with tap water followed by distilled water. After draining excess water, the sand was dried in an oven at 60o C overnight. Additionally, to eliminate the potential influence of microorganisms, all solutions, unwashed-sand and washed-sand were autoclaved at 121o C for 30 min. In experiments 2 and 3, auto-timers provided a day length of 14 h, mimicking the current seasonal photoperiod. Other modifications included extending the thin plastic sheet over each test plant to minimize bias due to transpirational losses from plants extending above the top of the beakers (*e.g.*, trees) as compared with those contained within the beakers (*e.g.*, submerged aquatic species).

The species assigned to each run are listed in Table 1, with the designated run number in parentheses following the common name. Plants were assigned to treatments randomly for all three experiments, with roots rinsed and plants weighed, as described above. The solution from each beaker was sampled daily at approximately 5 pm for the 10-day duration of each experimental run to determine perchlorate depletion and to identify the formation of any metabolites. Prior to sampling, contents of the beaker were mixed gently with a glass rod to collect a representative sample. A 1- mL sample of solution then was collected using a disposable pipette tip. The sample was filtered into a glass vial with a teflon-lined cap. Samples were refrigerated (5o C) in the dark until analyzed.

After sample collection on the final day, all plant material was removed from each beaker, rinsed in distilled water, and final wet weights determined for whole plants. Each plant then was separated into individual organs (i.e., leaves, roots, and stems and, when present, rhizomes). Exceptions included the following: pickleweed and perennial glasswort, which have scale-like leaves incorporated with the stem; waterweed, which has small, dense, whorled leaves fused with the stem at the base; and duckmeat, which was maintained intact because of the small size of the entire plant. The plant material was placed in aluminum packets and dried at 45o C in a drying oven for a minimum of 48 h to establish a constant weight. The dried plant material was weighed by organ type, and the total dry weight for each plant was determined for use in future calculations. The dried plant material was analyzed for perchlorate and its transformation products, as described in 3.5.2. Controls without perchlorate were included in each run, for treatments with and without sand. The controls were subjected to the same treatment as described above.

3.4 Tissue Extraction

To determine the perchlorate accumulation and formation of metabolites in plant tissues qualitatively, plants were selected from a single treatment, based on the depletion of perchlorate from solution. Dried plant organs (*e.g.*, roots, leaves and stems) from the selected treatments were ground separately into a powder using a mortar and pestle. Then the samples were transferred into 10 mL glass bottles containing 5 mL of 5-mM sodium hydroxide solution. The bottles were mixed by shaking briefly at regular intervals of approximately 6 h. After 48 h, each sample was transferred into a 2 mL vial and centrifuged at 7000 rpm for 10 min. Finally, the supernatant was transferred into an auto-sampler vial for analysis.

3.5 Analytical Methods

3.5.1 Analysis of Chloride, Perchlorate, and Metabolites

A Dionex Ion Chromatograph equipped with a gradient pump, UV detector, auto-sampler and auto-injector was used for analysis of chloride ions from the sorption experiment. The same equipment and analysis procedure were used to detect perchlorate in solution extracted during the kinetics experiments and the perchlorate metabolites in organs from plants that had been included in the kinetics experiments. No pretreatment of the samples was necessary with this method of analysis.

Ion analysis was performed with an Ionpac AS 11-HC (4-mm) analytical column. A guard precedes the analytical column to prevent sample contaminants from eluting onto the analytical column. The column was operated at 35^{o} C, and the flow rate of solvent (sodium hydroxide 100mM) was 1.0 mL min-1. The injection loop volume was 25 l, and the runtime for perchlorate analysis was 12 min. An anion self-regenerating suppressor (ASRS) was used for suppressed conductivity detection. Water as a mobile phase was used for regeneration of the ASRS. The detection limit of the analytical method was 10 ppb.

3.5.2 Tissue Analysis

Tissue samples were analyzed by ion chromatography using the procedure referenced above. An Ionpac AG9-HC analytical column with an AS9-HC guard column was used for the metabolites such as chlorate, chlorite and chloride ions. The mobile phase was 9mM sodium carbonate. The Ionpac AG9-HC provided an improved separation over the AS-11 HC column for trace ion analysis.

3.5.3 Data Analysis

The total accumulation plus degradation of perchlorate by the whole plant was assumed to be equal to the total depletion of perchlorate from solution at the end of the ten-day run (q). The value for uptake plus transformation was normalized by taking the measured value of solution depletion (q) and dividing by the wet weight of the plant at the end of the run. These normalized values are the total perchlorate sink in mg/kg.

First-order rate constants were determined by plotting the solution phase concentration time course-data as $\ln(C/C_0)$ vs. t. A non-linear regression analysis was completed, and the resulting slope of the line reported as the pseudo-first-order rate constant (k). The rate constants for each treatment were determined using this procedure.

4.0 RESULTS AND DISCUSSION

4.1 Adsorption of Perchlorate and Depletion Characteristics

The adsorption-desorption characteristics of perchlorate play an important role in the fate and transport in natural systems. The fate of this chemical is dependent on its sorption, which is thought to occur as a result of partitioning into soil or sediment, such as sand, by an ion-exchange process. Our lab-scale sorption results showed that 50-64% of the perchlorate originally in solution become adsorbed to sand, replacing an equivalent amount of the chloride ion formerly associated with the sand. Chloride ions displaced were related directly to the amount of perchlorate removed from solution. The displaced chloride poses a potential problem by inhibiting the perchlorate ion uptake by plant systems, and by causing chloride toxicity to sensitive species. The results of this preliminary adsorption experiment with perchlorate in unwashed-sand, and the apparent toxicity responses by some plants in Run 1, led to the expanded experimental design used in the subsequent two experiments specifically to evaluate the effects of washed and unwashed sand on perchlorate depletion from the solution in the presence of various plant species.

Representative depletion curves are provided in Figures 4-9, and additional characteristics of depletion of perchlorate from solution are provided in Tables 2-6. Total depletion of perchlorate from solution occurred in the presence of cabbage gum within eight days, while perchlorate in the "no-plant" control remained at 20.0 ppm for the unwashed-sand treatment without nutrients (Figure 4). The increase in concentration for the control at approximately 150 h was due to additional solution added at this time. For the same concentration of perchlorate in the presence of sweetgum for the unwashed sand treatment with nutrients, total depletion of perchlorate occurred after nine days (Figure 5). Seedlings and mature-wood cuttings of eastern cottonwood exhibited responses similar to the depletion curves of cabbage gum and sweetgum.

For the same treatment described in Figure 4, but with black willow, less than half of the concentration of perchlorate was depleted at the termination of the experiment on day ten (Figure 6). Species with depletion curves similar to black willow included green-wood cuttings of cottonwood and fragrant white water-lily. For the same treatment described in Figure 5, but with tarragon, only trace amounts of perchlorate remained in solution on day five of this experiment (Figure 7). Total depletion of perchlorate from the no-sand treatment without nutrients occurred by day seven, in the presence of parrot-feather (Figure 8). Concentrations of perchlorate in the control remained at 20.0 ppm. For the treatment with washed sand without nutrients at the same concentration of perchlorate, and in the presence of pickleweed, less than half of the perchlorate was depleted by day seven, when no solution remained for sampling (Figure 8). Smartweed and perennial glasswort had depletion curves similar to pickleweed.

A summary of perchlorate depletion from solution in the presence of plants tested is provided in Table 2. A summary of the percent of perchlorate depletion from solution in the presence of tested plants with positive results is provided in Table 3. Mass of perchlorate depletion for the species with positive results is summarized in Table 4. The values for the first-order kinetics are provided in Table 5 by treatment, and are summarized in Table 6. Water weed and duck meat are not included in Tables 3-5 because there was no depletion of perchlorate during the ten day experiment for these species.

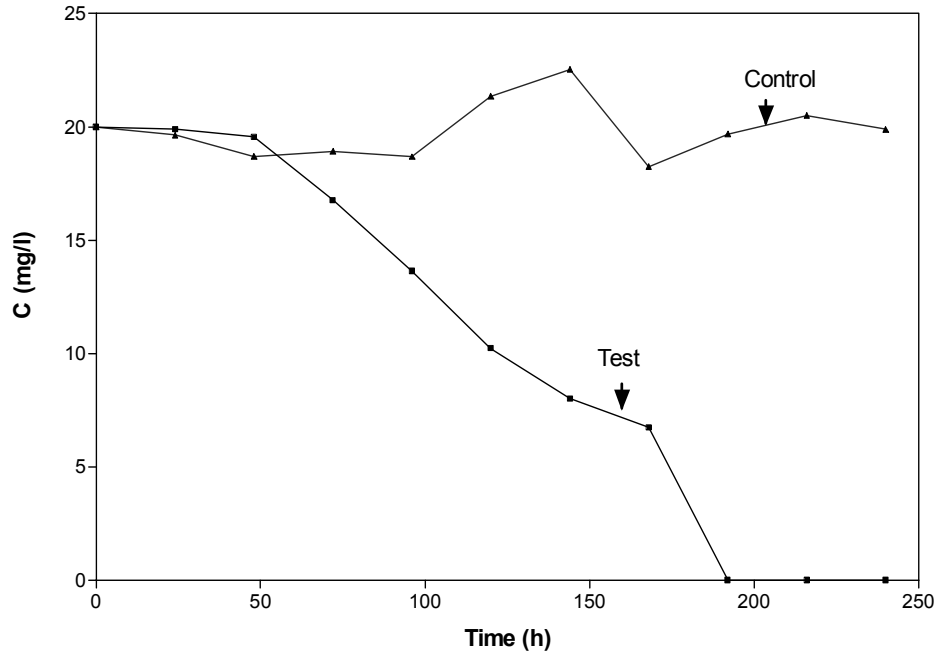

Figure 4. Depletion of Perchlorate (20 ppm) from Solution in the Presence of Cabbage Gum: Unwashed Sand + Deionized Water Treatment.

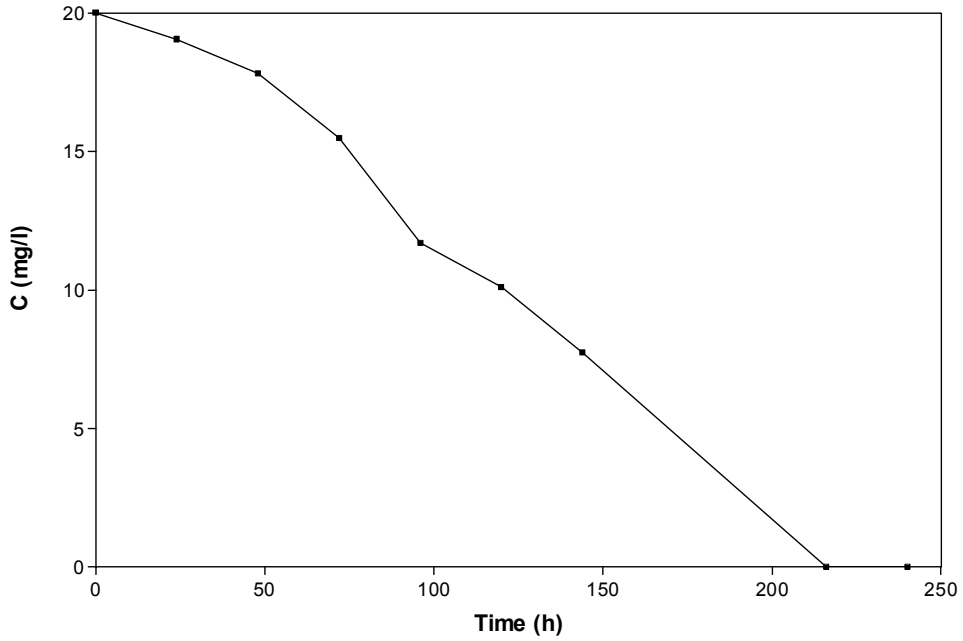

Figure 5. Depletion of Perchlorate (20 ppm) from Solution in the Presence of Sweetgum: Unwashed Sand + Nutrient Solution Treatment.

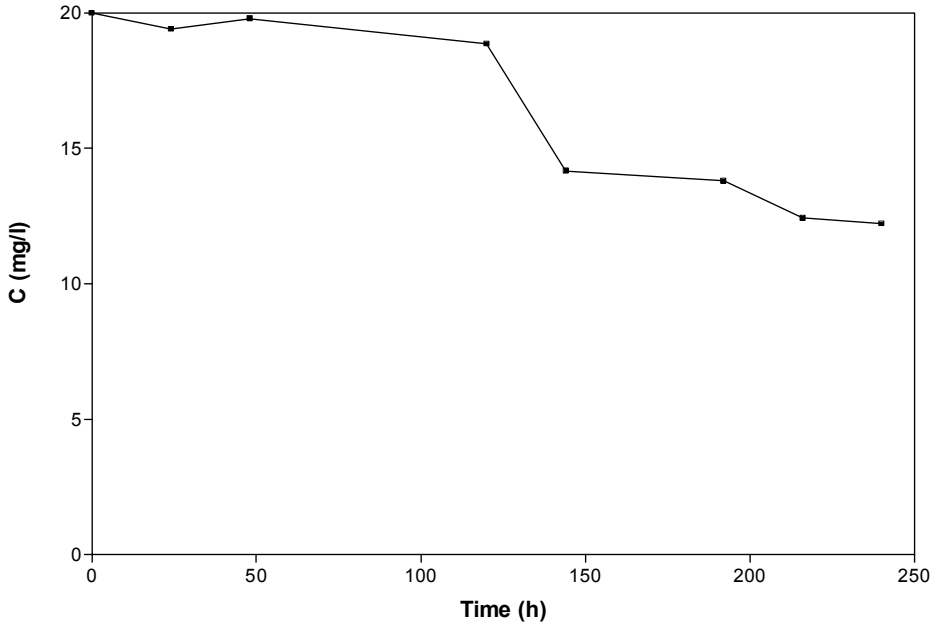

Figure 6. Depletion of Perchlorate (20 ppm) from Solution in the Presence of Black Willow: Unwashed Sand + Deionized Water Treatment.

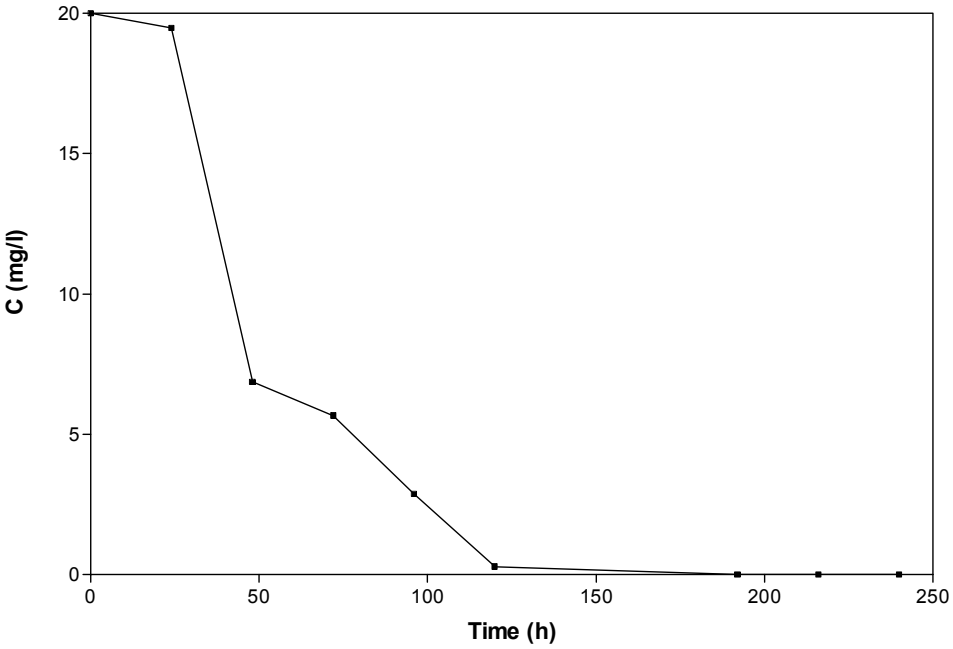

Figure 7. Depletion of Perchlorate (20 ppm) from Solution in the Presence of Tarragon: Unwashed Sand + Peters Solution Treatment.

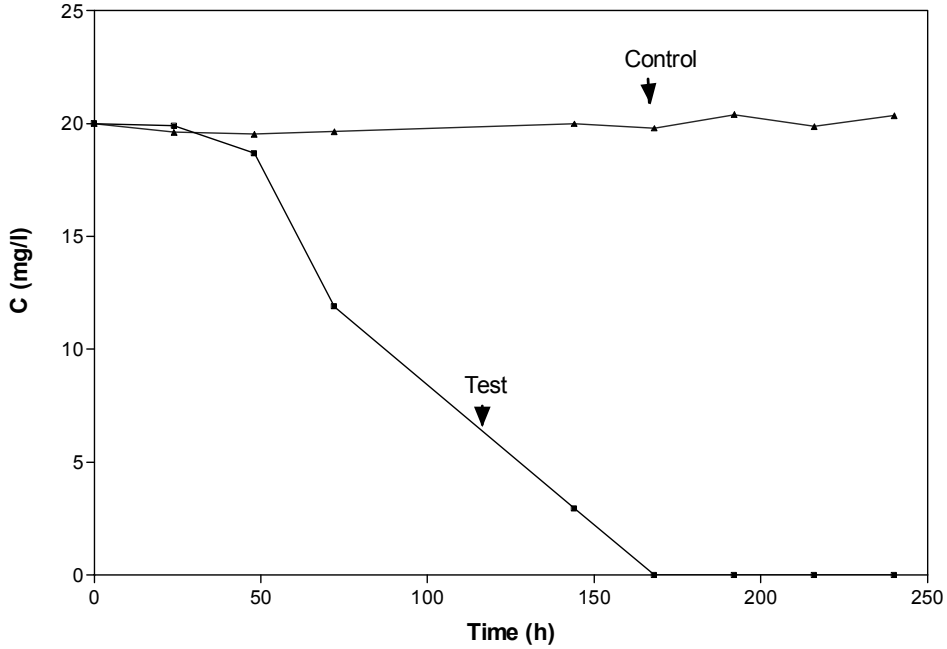

Figure 8. Depletion of Perchlorate (20 ppm) from Solution in the Presence of Parrot-feather: Deionized Water (No Sand) Treatment.

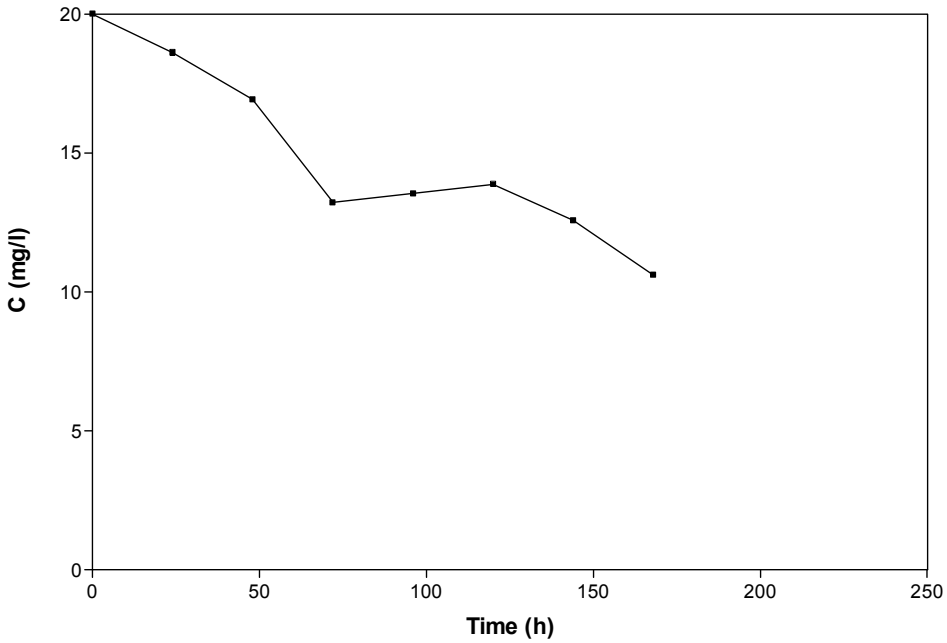

Figure 9. Depletion of Perchlorate (20 ppm) from Solution in the Presence of Pickleweed: Washed Sand + Deionized Water Treatment.

Table 2. Summary of Perchlorate Depletion from Solution in the Presence of Plants

	Unwashed Sand (w/ nutrients)			Aqueous Solution (w/ nutrients)			Washed Sand (w/ nutrients)	Washed Sand (w/o nutrients)	Unwashed Sand (w/o nutrients)
	Concentrations of Perchlorate (ppm)								
Plants	0.2	2.0	20.0	0.2	2.0	20.0	20.0	20.0	20.0
Trees									
Cabbage gum	✓	✓	✓	NT	NT	NT	✓	✓	×
Sweetgum	✓	✓	✓	×	×	×	✓	✓	×
Cottonwood									
(mature-wood)	✓	✓	✓	×	×	×	NT	NT	NT
(green-wood)	×	×	✓	NT	NT	NT	NT	NT	NT
Willow	✓	✓	✓	✓	✓	✓	✓	✓	✓
Upland Herbs									
Tarragon	✓	✓	✓	✓	✓	✓	NT	NT	NT
Wetland Herbs									
Pickleweed	×	×	✓	NT	NT	NT	×	✓	×
Blue-hyssop	✓	✓	✓	✓	×	×	×	✓	×
Smartweed	✓	✓	✓	×	×	×	×	✓	×
Glasswort	×	×	×	NT	NT	NT	✓	✓	×
Aquatic Herbs									
Waterweed	×	×	×	×	×	×	NT	NT	NT
Parrot-feather	✓	✓	✓	✓	✓	✓	NT	NT	NT
Water-lily	×	✓	×	×	✓	✓	NT	NT	NT
Duckmeat	×	×	×	×	×	×	×	×	×

✓ = moderate to high depletion of perchlorate
× = zero to low depletion of perchlorate
NT = not tested

Table 3. Percent of Perchlorate Depletion from Solution in the Presence of Plants

	Unwashed Sand (w/ nutrients)			Aqueous Solution (w/ nutrients)			Washed Sand (w/ nutrients)	Washed Sand (w/o nutrients)	Unwashed Sand (w/o nutrients)
	Concentrations of Perchlorate (ppm)								
Plants	0.2	2.0	20.0	0.2	2.0	20.0	20.0	20.0	20.0
Trees									
Cabbage gum	100	100	55	NT	NT	NT	100	100	0
Sweetgum	100	45	45	0	0	0	40	100	0
Cottonwood									
(mature-wood)	100	52	33	0	0	0	NT	NT	NT
(green-wood)	0	0	15	NT	NT	NT	NT	NT	NT
Willow	78	58	57	68	68	69	98	51	50
Upland Herbs									
Tarragon	100	100	100	100	100	100	NT	NT	NT
Wetland Herbs									
Pickleweed	0	0	29	NT	NT	NT	0	43	0
Blue-hyssop	100	36	55	87	0	0	0	29	0
Smartweed	100	65	47	0	0	0	0	54	0
Glasswort	0	0	0	NT	NT	NT	13	68	0
Aquatic Herbs									
Parrot-feather	100	100	97	100	100	100	NT	NT	NT
Water-lily	0	100	0	0	44	13	NT	NT	NT

NT = not tested

Table 4. Mass of Perchlorate Depletion from Solution in the Presence of Plants

	Unwashed Sand (w/ nutrients)			Aqueous Solution (w/ nutrients)			Washed Sand (w/ nutrients)	(w/o nutrients)	Unwashed Sand (w/o nutrients)
	Concentrations of Perchlorate (ppm)								
Plants	0.2	2.0	20.0	0.2	2.0	20.0	20.0	20.0	20.0
Trees									
Cabbage gum	6.5 (9.2)	53.5 (11.2)	**402** (8.2)	NT	NT	NT	**923** (6.5)	**674** (8.9)	0
Sweetgum	2.5 (23.2)	42.5 (14.1)	**145** (18.5)	0	0	0	**160** (6.5)	**340** (8.9)	0
Cottonwood									
(mature-wood)	3.2 (18.6)	13.1 (23.9)	44.5 (44.5)	0	0	0	NT	NT	NT
(green-wood)	0	0	**250** (3.6)	NT	NT	NT	NT	NT	NT
Willow	1.0 (15.1)	4.6 (24.8)	5.8 (19.8)	2.5 (16.6)	2.7 (14.3)	3.0 (13.9)	**120** (16.3)	73.1 (14.0)	46.4 (21.8)
Upland Herbs									
Tarragon	1.8 (11.0)	21.0 (9.1)	**162** (12.3)	5.3 (11.4)	59.4 (10.1)	**504** (11.9)	NT	NT	NT
Wetland Herbs									
Pickleweed	0	0	**305** (1.9)	NT	NT	NT	0	**614** (1.4)	0
Blue-hyssop	**120** (0.5)	**216** (1.0)	**6600** (0.5)	74.1 (0.7)	0	0	0	**1933** (0.9)	0
Smartweed	12.5 (4.8)	**150** (2.6)	**564** (5.0)	0	0	0	0	**981** (3.3)	0
Glasswort	0	0	0	NT	NT	NT	**780** (1.0)	**3138** (1.3)	0
Aquatic Herbs									
Parrot-feather	3.7 (5.4)	46.5 (4.3)	**392** (5.2)	11.8 (5.1)	**117** (5.1)	**1200** (5.0)	NT	NT	NT
Water-lily	0	**127** (4.7)	0	0	91.3 (2.9)	**312** (2.5)	NT	NT	NT

All values are mg/kg wet weight of plants; numbers in parenthesis are plant weight before initiation of experiments
NT = not tested

Table 5. First-order Kinetics of Perchlorate Depletion from Solution in the Presence of Plants*

	Unwashed Sand (w/ nutrients)			Aqueous Solution (w/ nutrients)			Washed Sand (w/ nutrients)	Washed Sand (w/o nutrients)	Unwashed Sand (w/o nutrients)
	Concentrations of Perchlorate (ppm)								
Plants	0.2	2.0	20.0	0.2	2.0	20.0	20.0	20.0	20.0
Trees									
Cabbage gum	0.008	0.002	0.005	NT	NT	NT	0.008	0.007	0
Sweetgum	0.003	0.002	0.007	0	0	0	0.003	0.007	0
Cottonwood									
(mature-wood)	0.013	0.004	0.010	0	0	0	NT	NT	NT
(green-wood)	0	0	0.007	NT	NT	NT	NT	NT	NT
Willow	0.004	0.003	0.003	0.005	0.004	0.003	0.003	0.001	0.002
Upland Herbs									
Tarragon	0.035	0.020	0.032	0.018	0.025	0.031	NT	NT	NT
Wetland Herbs									
Pickleweed	0	0	0.072	NT	NT	NT	0	0.17	0
Blue-hyssop	0.014	0.006	0.004	0.009	0	0	0	0.003	0
Smartweed	0.001	0.006	0.007	0	0	0	0	0.005	0.002
Glasswort	0	0	0	NT	NT	NT	0.216	0.22	0
Aquatic Herbs									
Parrot-feather	0.005	0.014	0.017	0.004	0.012	0.090	NT	NT	NT
Water-lily	0	0.004	0	0	0.002	0.001	NT	NT	NT

*First-order rate constant (k) in hr^{-1}

NT = not tested

Table 6. Summary of First-order Kinetics of Perchlorate Depletion from Solution in the Presence of Plants

Plants	Sand k (day^{-1})	Aqueous k (day^{-1})
Trees		
Cabbage gum	0-0.19	NT
Sweetgum	0-0.17	0
Cottonwood		
(mature-wood)	0.09-0.31	0
(green-wood)	0-0.17	NT
Willow	0.02-0.09	0.10
Upland Herbs		
Tarragon	0.48-0.77	0.43-0.74
Wetland Herbs		
Pickleweed	0-0.17	NT
Blue-hyssop	0-0.34	0.01
Smartweed	0-0.14	ND
Glasswort	0-0.22	NT
Aquatic Herbs		
Waterweed	ND	ND
Parrot-feather	0.12-0.41	0.09-2.1
Water-lily	0-0.09	0.05
Duckmeat	ND	ND

ND = no depletion
NT = not tested

4.2 Kinetics of Perchlorate Depletion from Solutions

Depletion was calculated as a first-order kinetics reaction, with individual values reported for each treatment in Table 5 for the11 species for which perchlorate depletion occurred. The values for k, the pseudo-first-order depletion rate constants in Table 5 are given in hr^{-1}. A summary of the pseudo-first-order kinetics (day^{-1}) for the depletion of perchlorate for all species is provided in Table 6. Values (day^{-1}) for washed and unwashed-sand treatments were in the range of 0-0.22 for cabbage gum, sweetgum, rooted green-wood cuttings of cottonwood, willow, pickleweed, smartweed, glasswort, and water-lily. Upper values for rooted mature-wood cuttings of cottonwood, blue-hyssop, and parrot-feather were 0.31, 0.34, and 0.41, respectively. The range for tarragon was 0.48-0.77.

4.3 General External Plant Responses and Transformation

The plant species evaluated in this study can be grouped into the following four categories, based on external responses associated with experimental treatments: A) favorable response, B) no response, C) moderate adverse response, D) severe adverse response (Table 7). Plants assigned to the first category generally exhibited new growth in the presence of perchlorate. Plants assigned to Category B generally exhibited no noticeable external change in the presence of perchlorate. Category C plants generally exhibited some type of adverse response such as wilting, which may be reversible. Plants assigned to the final category generally died, or exhibited some other severe response, such as permanent wilting. The general external responses of plants with positive results during the experiments are summarized in Table 8. Possible mechanisms for depletion of perchlorate from solution, based on plant responses during these experiments are described in Table 9. Analysis of plant tissues verified the presence of perchlorate and transformation metabolites, including chloride (Table 10). Radiolabeled chloride and a quantitative analysis of plant tissues could be used in future studies to determine the amount and source of chloride contained within the plants.

4.3.1 Favorable Response

Three species were included in Category A (Table 7). The halophyte, pickleweed produced new growth in all treatments of perchlorate tested. These responses may have been mediated by pretreatment acclimation with DI water that depleted internal concentrations of ions. Perennial glasswort, the other halophyte tested, also appeared to have favorable responses in all perchlorate treatments. The final species included in Category A was waterweed. Waterweed appeared to produce new growth in the presence of perchlorate; however, no depletion of perchlorate was observed in the presence of this species.

4.3.2 No Response

Duckweed also was excluded from Table 8, for the same reason as waterweed, but was included in Category B (Table 7), because it exhibited no apparent external response to any of the treatments. Other species assigned to this category included black willow, blue-hyssop, and fragrant white water-lily. However, depletion of perchlorate occurred in the presence of these latter three species. The lack of any response by blue-hyssop, fragrant white water-lily, and duckmeat suggests that these species are tolerant of perchlorate and chloride, at least on a short-term basis. Blue-hyssop may be sensitive to excess nutrients (Table 8). Although black willow was assigned to this category, it exhibited severe adverse responses at lower concentrations of perchlorate in treatments with unwashed sand. These responses were attributed to a sensitivity to chloride ions displaced from the unwashed sand by perchlorate, and a complex interaction of higher concentrations of perchlorate in this species.

Table 7. Categorization of Plant Species Based on General External Responses to Perchlorate/Nutrient Treatments During Ten-Day Experiments

Scientific Names	Common Names
A - Favorable Response	
Allenrolfea occidentalis	pickleweed
Salicornia virginica	perennial glasswort
Elodea canadensis[1]	waterweed
B - No Response	
Salix nigra[1]	black willow
Bacopa caroliniana[1]	blue-hyssop
Nymphaea odorata	fragrant white water-lily
Spirodela polyrhiza	duckmeat
C - Moderate Adverse Response	
Eucalyptus amplifolia [2]	cabbage gum
Populus deltoides	eastern cottonwood
seedlings [2]	
mature-wood cuttings [1]	
green-wood cuttings [1]	
Polygonum punctatum [4]	smartweed
Myriophyllum aquaticum [4]	parrot-feather
D - Severe Adverse Response	
Artemisia dracunculus sativa[5]	tarragon
Liquidambar styraciflua[5]	sweetgum
Salix nigra[3]	black willow

[1] possible favorable response to perchlorate
[2] possible unrecoverable sensitivity to chloride ions/some sensitivity to perchlorate
[3] unrecoverable sensitivity to chloride ions
[4] recoverable sensitivity to chloride ions
[5] at least partially related to experimental design

Table 8. General External Responses of Plants with Positive Results During Ten-Day Perchlorate/Nutrient Experiments

Plants	External Responses				
	Unwashed Sand (w/ nutrients)	**Aqueous Solution** (w/ nutrients)	**Washed Sand** (w/ nutrients)	(w/o nutrients)	**Unwashed Sand** (w/o nutrients)
Trees					
Cabbage gum	lower leaves dead at all conc.	-	lower leaves dead	lower leaves dead	lower leaves dead
Sweetgum	wilted at all conc.	leaves wilted/dead at 20 ppm; lower conc. OK	leaves wilted/dead	leaves epinastic	leaves wilted/dead
Cottonwood					
seedling	decline decreases w/conc.	good at all conc.[2]	lower leaves dead	lower leaves dead	lower leaves dead
mature-wood[1]	decline decreases w/conc.	higher conc. ok	lower leaves dead	lower leaves dead	lower leaves dead
green-wood[1]	decline increases w/conc.	-	-	-	-
Willow	dead at lower conc.[3]	good at all conc.	good	good	dead
Herbs					
Tarragon	decline increases w/conc.[4]	shriveled, dead[5]	-	-	-
Pickleweed	robust/new growth all conc.[5]	-	new growth	new growth	new growth
Blue-hyssop	OK	decaying at 0.2 ppm	OK	OK	OK
Smartweed	decline increases w/conc.	decline decreases w/conc.	lower leaves dead	lower leaves dead	leaves wilted/dead
Glasswort	OK	-	OK	OK	OK
Parrot-feather	wilted at lower conc.[5]	robust at all conc.	-	-	-
Water-lily	OK	OK	-	-	-

1 green-wood cuttings from Carswell AFB, with **possible fungal infections**
2 possible biochemical change w/nutrients only and nutrients with 20.0 ppm perchlorate attracting aphids
3 **wilted** at 20.0 ppm perchlorate **(chloride toxicity?)**
4 **black stain in sand (exudates?)** for 2.0 and 20.0 ppm perchlorate
5 **dark red stain (tissue/cellular degradation?)** in solution for all concentrations of perchlorate
6 no influence on perchlorate at 0.2 or 2.0 ppm perchlorate
7 signs of **recovery** at Day 10

Table 9. Possible Mechanisms for Depletion of Perchlorate from Solution Based on Plant Responses During Ten-Day Experiments [1]

Trees:	Possible Mechanisms:
cabbage gum (*Eucalyptus amplifolia*)	B, **C**, D, E
sweetgum (*Liquidambar styraciflua*)	B, **C**, D, **E** [2]
eastern cottonwood (*Populus deltoides*)	
seedlings	B, **C**, D, **E** [2]
mature-wood cuttings [4]	B, **C**, D, **E** [2]
green-wood cuttings [4]	B, C, D, **E** [2]
black willow (*Salix nigra*)	**A** [3], B, **C**, D, **E** [2]
Upland Herbs:	
tarragon (*Artemisia dracunculus sativa*)	**A** [3], **B** [5],**C**, D, **E** [2]
Wetland Herbs:	
pickleweed (*Allenrolfea occidentalis*)	**A** [3], **B** [5], **C**, D, E
blue-hyssop (*Bacopa caroliniana*)	**A**, B, **C**, D, **E** [2]
smartweed (*Polygonum punctatum*)	**A**, B, **C**, D, **E** [2]
perennial glasswort (*Salicornia virginica*)	B, **C**, D, E
Aquatic Herbs:	
waterweed (*Elodea canadensis*)	no depletion
parrot-feather (*Myriophyllum aquaticum*)	B, **C**, D, **E** [2]
fragrant white water-lily (*Nymphaea odorata*)	**B** [5], **C**, D, E
duckmeat (*Spirodela polyrhiza*)	no depletion

[1] see Figure 10 for explanation of A-D; E = adsorption to sand; bold codes indicate observed responses that suggest that mechanism

[2] visible response of plant in **unwashed sand** at certain concentrations suggesting chloride ion interaction

[3] may be **short-term only**, as plant dies/decomposes

[4] rooted cuttings from Carswell Air Force Base, possibly with **fungal pathogens**

[5] dark **staining** observed

Perchlorate, intermediate metabolites (chlorate, chlorite), and the end product chloride were documented in black willow organs (Table 10), confirming uptake by this species. However, a determination cannot be made from these data regarding whether the source of chloride was from transformation of perchlorate, or from chloride ions displaced from the sand. Experiments with labeled chloride are needed to resolve this question. If black willow is capable of transforming perchlorate to the end product chloride, additional long-term experiments should be conducted to evaluate the sensitivity of black willow to increasing concentrations of chloride ions. Additional complications could arise if this species is used at a site where chloride is associated with the soils at the site, and the chloride ions are displaced by perchlorate and taken up by the plant. Finally, additional experiments should be conducted to determine if seedlings, saplings, and rooted cuttings obtained from other sources respond similarly to the rooted cuttings from Carswell used in these experiments, since the latter may have had additional factors involved (*e.g.*, fungal pathogens), which contributed to low rooting success.

4.3.3 Moderate Adverse Response

Species assigned to Category C, moderate adverse responses to treatments, included cabbage gum, eastern cottonwood seedlings, mature-wood cuttings, and green-wood cuttings, smartweed, and parrot-feather. In cabbage gum, the lower leaves of plants in all treatments died initially (Table 7). Perchlorate and all metabolites were detected in cabbage gum roots. Stems contained perchlorate and chlorate. Leaves contained only chlorate and chlorite (Table 10). Cabbage gum may be experiencing mild chloride toxicity or may be responding adversely to the saturated conditions of the treatments.

Responses in cottonwood seedlings varied by treatment. No adverse response was observed in the no-sand treatment with nutrients. However, the lower leaves died in all other treatments (Table 8). In the washed-sand treatment with nutrients, seedling decline decreased with increasing concentration of perchlorate. These responses suggest that cottonwood seedlings are tolerant of saturated soil conditions, sensitive to chloride ions, but may be able to take up perchlorate preferentially, when both perchlorate and chloride are in solution. All of the tissue that was analyzed (4c) from the cottonwood seedling organs (root, stem and leaf) contained chloride. Therefore, if the source of the tissue chloride was from transformation of perchlorate, long-term exposure to perchlorate could lead to chloride toxicity.

Rooted mature-wood cuttings of cottonwood responded similarly to cottonwood seedlings, with adverse impacts declining with higher concentrations of perchlorate. Perchlorate and all metabolites were found in roots and the woody stem of the rooted mature-wood cuttings of cottonwood that were analyzed (1c). Chlorate, chlorite, and chloride were found in leaf tissue of the same sample (Table 10). Therefore, the same potential for chloride toxicity via accumulation from perchlorate transformation exists for these cuttings. The presence of these metabolites in seedling tissues suggests that perchlorate is being transformed within, or translocated through all organs. The same conclusion cannot be made for cottonwood cuttings without analysis of control plants that were not exposed to experimental concentrations of perchlorate, since the cuttings were obtained from a site contaminated with perchlorate.

Rooted green-wood cuttings of cottonwood in washed-sand with nutrients responded differently than cottonwood seedlings, with adverse impacts increasing with higher concentrations of perchlorate. Other treatments were not included for green-wood cuttings because of the limited plant material. Additional research is required to determine the reason for the differences in these responses for different-aged tissue of cottonwood.

Table 10. Metabolites of Perchlorate Transformation Identified in Plant Tissues

Plants[1]	Metabolites		
	Roots	**Leaves**	**Stems[2]**
Cabbage gum (3c)	perchlorate chlorate chlorite chloride	chlorate chlorite	perchlorate chlorate
Sweetgum (3c)	perchlorate chlorate chlorite chloride	perchlorate chlorite	perchlorate chlorate chlorite chloride
Cottonwood (seedlings)(4c) (mature-wood)(1c)	perchlorate chlorate chlorite chloride	chlorate chlorite chloride	perchlorate chlorate chlorite chloride
Willow (5c)	perchlorate chlorate chlorite chloride	 chlorate chlorite	perchlorate chlorate chlorite chloride
Tarragon (1c)	perchlorate chlorate chlorite	 chlorate chlorite	perchlorate chlorate chlorite
Pickleweed[2] (4c)	perchlorate chlorate chlorite chloride	perchlorate chlorate chlorite chloride	
Blue-hyssop (4c)	perchlorate chlorate chloride	perchlorate chlorate chlorite chloride	perchlorate chlorate chlorite
Smartweed (1b)	perchlorate chlorate chlorite	perchlorate chlorate chlorite	perchlorate chlorate chlorite
Glasswort[2,3] (4c)	chlorate chloride	chlorate chlorite chloride	perchlorate chlorate chlorite
Parrot-feather (1c)	perchlorate chlorate chlorite chloride	perchlorate chlorite chloride	perchlorate chlorate chlorite
Water-lily[3] (2b)	perchlorate chlorate chlorite chloride	perchlorate chlorate chloride	chlorate chlorite chloride

[1] treatment code for sample provided in parenthesis

[2] leaves and stems fused, analyzed as a single tissue, and reported as leaves

[3] metabolites in rhizomes are reported as stems

Responses of smartweed also varied with treatment. Similar responses were observed in unwashed-sand treatments without nutrients, and in washed-sand with, and without nutrients (Table 8). In unwashed-sand treatments with nutrients, plant decline increased with increasing concentration of perchlorate, similar to the response in green-wood cuttings of cottonwood. The reverse occurred in the no-sand treatment with nutrients. Perchlorate and chlorate were found in roots, stems, and leaves of smartweed, suggesting that perchlorate is being transformed within, or translocated through all organs (Table 10). More research is required to analyze the complexity of responses observed in smartweed.

4.3.4 Severe Adverse Response

The three species included in Category D were sweetgum, black willow, and tarragon (Table 7). Sweetgum responded adversely to all treatments, except lower concentrations of perchlorate in no-sand treatments (Table 8). No recovery from wilting was observed. The response in no-sand treatments compared to treatments with sand suggest that chloride toxicity may be responsible, in part, for the responses, and the treatments with washed-sand may have contained residual chloride. A random test of sand from washed-sand treatments at the conclusion of the final experiment revealed approximately 0.2 mg/L of residual chloride. The source of the chloride presumably was the sand, although experiments with labeled chloride are required for an accurate determination. For the sweetgum tissue selected for analysis (3c), perchlorate and all metabolites were found in roots and stems, but leaves contained only perchlorate and chlorite (Table 10).

Black willow appeared to have an extreme sensitivity to chloride, as indicated by plants dying in the unwashed-sand treatments (Table 8). Consequently, if perchlorate metabolites are accumulated in black willow or if chloride is present at the treatment site, chloride toxicity could become a problem with this species. Tissue analysis (5c) indicated that perchlorate and all metabolites were present in the roots and woody stems of black willow. Leaves contained only chlorate and chlorite (Table 10).

Tarragon died by the end of the experiment in both no-sand and sand treatments, with decline increasing with increasing concentrations of perchlorate (Table 8). Both perchlorate and chloride are thought to be factors in tarragon's response. An analysis of plant tissue (2c) confirmed the presence of perchlorate, chlorate, and chlorite in roots and stems of tarragon, while leaves contained only chlorate and chlorite (Table 10).

4.4 Predicted Field-Scale Performance and Possible Mechanisms for Perchlorate Depletion

One important consideration for field performance of potential phytoremediation candidates is the mass of perchlorate that can be removed by the selected plant. An arbitrary ranking system was developed for general comparison of phytoremediation potential based on the mass of perchlorate depleted from solution per mas of plant species tested (mg/kg wet weight). The five categories are as follows: 0 = no depletion; 1-99 = minimal depletion; 100-499 = moderate depletion; 500-999 = moderately high depletion; and >1000 = high depletion. None of the tree nor the herbaceous, upland species tested were included in the highest category of performance (Table 4). Wetland and aquatic plants included in the highest category were blue-hyssop for the 20.0 ppm perchlorate treatments with unwashed-sand and nutrients (6600 mg/kg), and washed-sand without nutrients (1933 mg/kg); perennial glasswort for the 20.0 ppm perchlorate treatment with washed-sand without nutrients (3138 mg/kg); and parrot-feather for the 20.0 ppm perchlorate no-sand treatment with nutrients (1200 mg/kg).

Results in the moderately-high category were obtained for one tree species, the herbaceous upland species, and three of the four herbaceous wetland species evaluated, all at 20.0 ppm

perchlorate (Table 4). For cabbage gum, the mass of perchlorate depleted was moderately high for the washed-sand treatment with nutrients (923 mg/kg) and without nutrients (674 mg/kg). For tarragon, the mass of perchlorate depleted was moderately high for the no-sand treatment with nutrients (504 mg/kg). For pickleweed, the mass of perchlorate depleted was moderately high for the washed-sand treatment without nutrients (614 mg/kg). For smartweed, the mass of perchlorate depleted was moderately high for the unwashed-sand treatment with nutrients (564 mg/kg) and the washed-sand treatment without nutrients (981 mg/kg). Finally, for glasswort, the mass of perchlorate depleted was moderately high for the washed-sand treatment with nutrients (780 mg/kg).

Results in the moderate category (100-499 mg/kg) also are shown in bold in Table 4. In summary, depletion of perchlorate appears to be influenced by plant species, perchlorate concentration, sand versus no-sand treatments, the presence or absence of nutrients, the age of plant tissue, and by the presence of chloride ions. A discussion of the predicted field scale performance of each species is provided in the following subsections.

Four possible mechanisms for the potential fate of perchlorate in plant systems are shown in Figure 10. The first two mechanisms, (A and B) involve external degradation/transformation, while the following two mechanisms (C and D) involve internal degradation/transformation after uptake of perchlorate by the plant. Mechanism A occurs in dead or dying plants as tissues are degraded and cell contents are released. Mechanism B involves substances exuded from live plants (*e.g.*, root exudates). Mechanism C involves the internal transformation of perchlorate without accumulation of perchlorate or metabolites, while mechanism D involves internal transformation with accumulation of perchlorate or metabolites (*e.g.*, chloride). A fifth mechanism that is physical rather than biological (E), also could be responsible for depletion of perchlorate. This mechanism involves exchange of perchlorate for chloride adsorbed to the sand. The possible mechanisms for perchlorate depletion from solutions are provided in Table 9. Codes printed in bold indicate observed responses that suggest that mechanism.

Samples for analysis of tissue samples were selected based on the maximum reduction in solution perchlorate concentration, for each of the 11 species for which perchlorate depletion was observed. Tissues of individual plant organs (*e.g.*, roots, stems, leaves) from the selected treatments were analyzed. Perchlorate, or transformation metabolites (chlorate, chlorite, and chloride) were observed in all tissues analyzed (Table 10). Future work should include: 1) a quantitative analysis of individual plant organs (*e.g.*, roots, stems, leaves), and 2) labeled chloride to determine the amount and source of chloride contained within the plants.

4.4.1 Trees

4.4.1.1 Cabbage Gum

As indicated previously, one of the Air Force's considerations for plants to be used for phytoremediation is wood biomass production to defray costs of remediation. Production of Eucalyptus species was investigated in the 1960's and 1970's as a short-rotation woody crop for Alabama, Florida, Georgia, Louisiana, North Carolina, and Texas, but the effort was abandoned because of several problems. These problems included wood that was very brittle, leaves that contained high concentrations of volatile oils (making the trees highly flammable), and sensitivity to cold damage. Renewed interest in Eucalyptus species is due to improved genetic stock that is less susceptible to cold damage. Frost hardiness reportedly has been developed in *E. grandis*, so that it may survive at low temperatures (Don Rockwood, University of Florida, pers. comm., 7/13/98).

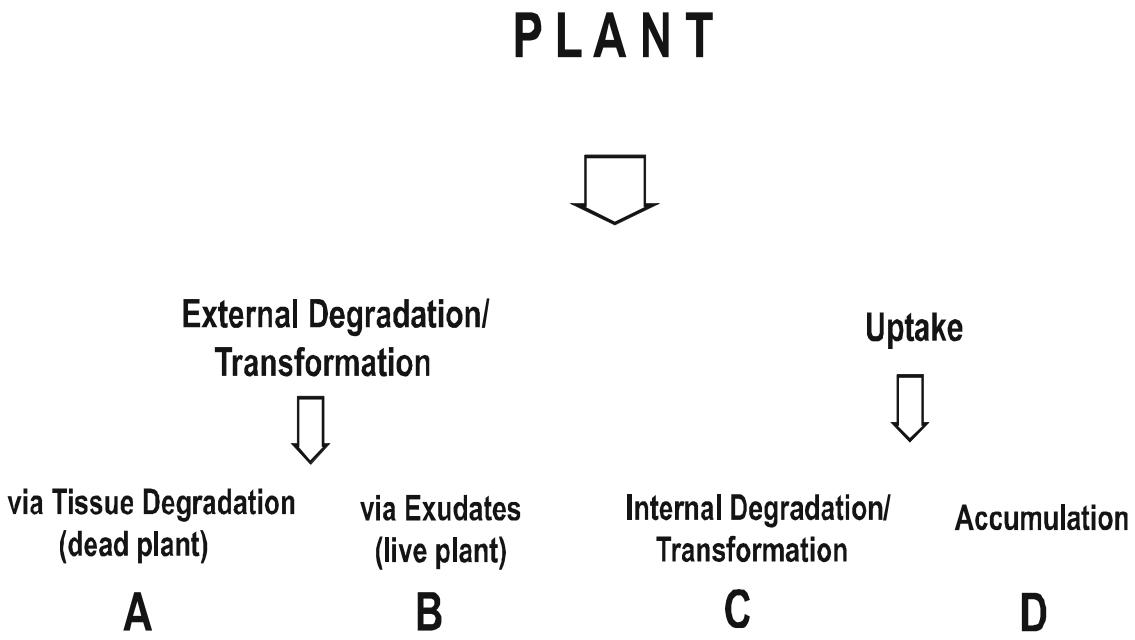

Figure 10. Potential Fate of Perchlorate in Plant Systems.

One of the primary concerns expressed by various sources in Florida is that Eucalyptus species, which have been imported from Australia, have escaped from these early "production" sites, and have invaded natural habitat. Reference to the invasive ability of these species is made by Wunderlin (1997) and by the Florida Exotic Pest Plant Council. Wunderlin (1997) has documented invasion by *E. grandis* and *E. robusta* in central and south Florida. The Council lists *E. camaldulensis* as a "Category II" plant ("species that have shown a potential to invade and disrupt native plant communities"). From a long-term perspective, consideration should be given to the liability associated with promoting non-native species that can 1) increase fire hazards in commercial and residential areas, and 2) result in additional costly, and possibly irreversible environmental damage in addition to the perchlorate contamination problem. Cabbage gum is not recommended for additional experiments since affected leaves did not recover during these experiments, new growth was not observed, and because of the adverse factors referenced above.

4.4.1.2 Sweetgum

Generally, sweetgum is tolerant of a wide range of growing conditions, particularly soil moisture content. This species occurs naturally in floodplain wetlands, and should be adapted to phytoremediation sites requiring roots to be in contact with ground water. The relatively poor performance of sweetgum in this project may have been due to the large root mass of the plants (largest of the plants tested) and the small size of the experimental containers. On-site or *in situ* experiments are recommended for further evaluation of the species.

4.4.1.3 Eastern Cottonwood

Four of the possible causal factors are discussed below to explain the small percentage of cottonwoods that produced roots and leaves during our experiments. More extensive experiments with cottonwood are required before the response of this species to perchlorate can be determined and predictions can be made regarding the potential field performance of this species.

Light:

The rooting treatments for the cottonwood (and willow) were conducted under artificial growth lights in the laboratory where the perchlorate exposure experiments were conducted. When the first experiment was conducted, lights in the laboratory were on continuously. The study design was modified for the second set of experiments to provide a more natural photoperiod (7 am to 9 pm). Either the initial, extended photoperiod, or the artificial light conditions, may have resulted in some type of disruption of the normal rooting mechanism for the cuttings. However, root and shoot growth under natural lighting (but different rooting conditions) in the greenhouse did not produce more favorable results. Additionally, another researcher also received cuttings from the same source and placed them outside in natural light to root (Valentine Nzengung, University of Georgia, pers. comm. 7/17/98). None of those cuttings produced roots.

Diameter/Age of Woody Tissue:

The majority of the cottonwood cuttings were large-diameter branches. The diameter of the branch tips and the appearance of the wood confirmed this plant material was older wood than cuttings produced from the branch tips, representing the current year's growth. Wood from the current year generally is thought to produce roots more readily. However, only approximately 15% of the smaller-diameter, green-wood cuttings from the Carswell AFB site produced roots and leaves.

Timing of Cuttings:

The cuttings for these experiments were taken in the middle of the summer. Timing of cuttings reportedly can be a factor influencing success of rooting (Kerry Britton, USDA Forest Service, pers. comm., 8/31/98). The lack of success in rooting the cuttings may have been due to seasonal factors.

Fungal Pathogens:

One of the cottonwood cuttings (10B) that was assigned randomly to the sand bed treatment in the EPA laboratory began exhibiting red-orange protuberances in the lenticels on July 11, 1998. By July 13, 1998, the protuberances had expanded, and a fungal infection was suspected. The cutting was removed from the rooting bed, enclosed in a protective wrap and transported to the USDA Forestry Sciences Laboratory in Athens, Georgia. No similar signs were observed in any other cuttings in the laboratory, or in the greenhouse at that time.

The next morning, Dr. Paula Spaine, Forest Pathologist with that laboratory, provided the results of her evaluation of the cottonwood cutting and substance associated with the lenticels. She indicated that the cutting had predisposing cankers (scar tissue from an earlier infection). She also indicated that the red-orange substance was ascospores (fruiting bodies) of the opportunistic fungal pathogen, *Cytospora.* She had not encountered this secondary pathogen prior to examining the plant material from Carswell AFB; however, the taxonomic reference she was consulting stated that *Cytospora* infects plants that are predisposed by stress (Sinclair *et al*., 1987). She also indicated that pruning the trees while they are under stress, and in the presence of pathogens may increase infection. She suggested that the remaining cuttings without roots and leaves be examined the following week by another Forest Pathologist with the USDA Forest Service, Dr. Kerry Britton, upon her return. Dr. Britton who conducted research on cottonwood, confirmed Dr. Spaine's conclusions. She added that *Cytospora* probably was not the cause of the poor rooting success and probably would not contribute to the decline of the trees if the trees were not predisposed by some unidentified stressor.

Plants can experience stress if planted outside their naturally occurring range. Therefore, an effort was made to determine the origin of the cottonwood trees that had been planted at Carswell AFB. The original 240 cottonwood trees planted at the AFB are "Sioux Land" variety and were supplied by Gandy Nursery in Ben Wheeler, Texas. The cottonwood trees were rooted from greenwood (branch tip) cuttings obtained from a natural population in the vicinity of the nursery, which is approximately two hours from the Carswell AFB site (Dennis Gandy, Gandy Nursery, pers. comm., 6/18/98). The proximity of the parent material to the AFB site suggests that the trees grown from the cuttings should be adapted to climatic conditions at Carswell AFB. However, site-specific soil, or soil moisture conditions at the AFB may not be optimal for cottonwood growth. After the trees were planted at the AFB, approximately 28 of the trees were gnawed-down by beavers. However, all of these trees resprouted (Greg Harvey, Wright Patterson AFB, pers. comm., 6/18/98). This is an additional factor that may have contributed to the poor rooting response if the cuttings were taken from resprouted trees. The stress which is predisposing the trees may be a natural phenomenon. Another possibility is that some site-specific condition at Carswell AFB, such as components in the ground water, may be predisposing these trees to infection by opportunistic fungal pathogens.

Several days after the cottonwood cuttings arrived and selected cuttings were placed in the tanks with aerated DI water, a tan-colored gelatinous substance was observed exuding from the

bases of some of the cuttings. A similar gelatinous substance, referred to as "gummosis" (production of a thick, dark, gummy substance) has been reported in peach trees subjected to water stress and infected by another opportunistic pathogen, *Botryosphaeria* (Brown and Britton, 1986). The gelatinous substance associated with some of the cottonwood cuttings may have been related to a fungal pathogen they were harboring. Some of the substance was collected for future analysis.

Similar gelatinous exudates were not observed associated with the cottonwood cuttings in the sand bed in the laboratory. However, when the cuttings were removed from the sand bed in the greenhouse on July 15, 1998, there were signs that some type of exudate was associated with many of the cuttings. The signs included circular zones of dark green algae and a gelatinous sheen on the surface of the sand around the base of many of the cottonwood cuttings. These zones extended approximately 1 to 2 cm and the algal growth appeared to be supported by these exudates. The zone were photographed for future analysis.

The cuttings that produced the most dramatic responses were small segments of mature-wood cuttings (approximately 3 cm in length), that were the residuals from the cuttings selected for rooting in the laboratory and for use in the final experiment. The diameter of these cuttings was approximately 2 cm. These segments had been placed vertically into the sand, with the upper surface approximately even with the surface of the sand. Similar exudate zones appeared around both ends of the smaller diameter cuttings (1 cm, and 4 cm in length) from the same experimental batch that were pushed into the sand horizontally. Similar stained zones also were observed around the bases of some of the green-wood cuttings that were less than 0.5 cm in diameter.

Based on the evidence of past and possible current infection of the cottonwood trees at the Carswell AFB site, and the long-term interest in cottonwood as a phytoremediation species and a biomass producer, the following recommendations are made. No additional cuttings should be taken from the Carswell AFB trees, based on recommendations of Forest Pathologists. The trees should be evaluated by a Forest Pathologist knowledgeable about secondary fungal pathogens and stressors in an attempt to identify whether the cottonwood (and willow) trees currently exhibit any symptoms of stress and, if so, what causal factors can be identified. On-site research at Carswell AFB should be initiated to investigate the degree to which perchlorate, or breakdown products actually occur in the tissues of the cottonwood and willow trees on-site.

4.4.1.4 Black Willow

Typically, willows are propagated readily from cuttings, and grow vigorously, producing considerable biomass in a short time. These characteristics make willow a prime candidate for on-site and *in situ* phytoremediation. Unfortunately, rooting success was low for the willow obtained for these experiments, and sensitivity to chloride ions was observed. The performance of black willow may be enhanced in the field. However, the susceptibility of this species to chloride toxicity and secondary pathogens should be investigated.

4.4.2 Upland Herbs

Total depletion of perchlorate from solution at all three concentrations tested occurred in the presence of tarragon (Table 3). The depletion of perchlorate from solution with this species occurred relatively rapidly in both unwashed sand with nutrients (Treatment 1) and no-sand with nutrients (Treatment 2) in the first run of these experiments (Table 5). The pseudo-first-order kinetic rates were comparable for the two treatments (Table 6), with total depletion occurring after

approximately five days for the greatest concentration (20 ppm) of perchlorate (Figure 7).

Mass of perchlorate depleted in the presence of tarragon (Table 4) was in the moderate range for Treatment 1(162 mg/kg wet weight), and the moderately high range for Treatment 2 (504 mg/kg wet weight). When compared to the mass of perchlorate depleted for the same concentration in Treatment 1, in the presence of trees, cabbage gum, sweetgum and green-wood cuttings of eastern cottonwood also were in the moderate range. When compared to the mass of perchlorate depleted for the same concentration in Treatment 1 in the presence of other herbaceous species, pickleweed and parrot-feather also were in the moderate range, and blue-hyssop was ranked high. For the no-sand treatment with nutrients at the same concentration (Treatment 2), water-lily also was ranked moderately high, and parrot-feather was ranked high (Table 4).

Washed-sand treatments were not incorporated in the first run of these initial perchlorate experiments when tarragon was tested in order to evaluate the degree to which exchange or adsorption of perchlorate might be occurring with unwashed-sand. However, depletion of perchlorate was greater in the no-sand treatment. These similar results in treatments with and without the unwashed-sand suggest that actual transformation of perchlorate was occurring in addition to any adsorption of perchlorate to the sand that may have occurred in Treatment 1 (Table 4).

Eventually, the bases of all of the stems of the tarragon that were tested, as well as those being acclimated in no-sand treatments, decayed and the plants died. Because tarragon was used in Run 1, washed-sand treatments were not included. The tarragon in the sand treatments may have been responding to chloride ions displaced from the unwashed-sand. However, tarragon plants transplanted into containers with the similar sand for use in future experiments, did not exhibit similar symptoms. Consequently, it appears that the plants in the sand-based treatments may have been responding to water-logged conditions. Some of the plants in the sand-based treatments had zones of black stain around the roots, which may have been exudates in response to the treatments. However, they may have been responding to water-stress by being contained in the undrained beakers.

The staining and tissue degradation are suggestive of external transformation processes A and B (Figure 10). The fact that tarragon exhibited a severe adverse response to the treatments (Table 7), and plant decline intensified as the concentration of perchlorate increased (Table 8) suggests that uptake also may be occurring in the form of internal transformation (C) or, more probably, tissue accumulation (D) that is debilitating or toxic to the plant. More detailed experimentation and tissue analysis are required to identify which of these mechanisms is the primary factor responsible for the depletion of perchlorate.

Whichever mechanism is responsible for perchlorate depletion in the presence of tarragon, this species appears to have limited tolerance for high soil moisture and no tolerance for standing water. Therefore, under field conditions the roots of tarragon probably would avoid contact with the water table when possible, relying on infiltration from rain (in well-drained soil) as the preferred source of water.

The sensitivity of tarragon to water-logged soil may limit the usefulness of this species for on-site phytoremediation of groundwater contaminants. It is possible that large stands of tarragon plants placed in well-drained areas over contaminated sites might leach exudates into the soil, and the exudates could be transported vertically via infiltration into the ground water. However, it appeared that the exudates observed in the initial experiments were being produced in response to the water stress. Consequently, production of the exudates may be limited if the plants are not forced, artificially, to be in contact with water-logged soils.

Although tarragon may not be well-suited for on-site phytoremediation of ground water

contaminated with perchlorate, treatment of the contaminated water may be possible using a flow-through system. In this case the contaminated ground water might be pumped from the ground, exposed to tarragon plants or extracts from tarragon tissue, then reinjected into the ground. This approach is more energy-intensive than using plant species that can be planted on-site for phytoremediation, but may be desirable under certain conditions.

Finally, several attempts were made to propagate tarragon vegetatively by rooting cuttings. This would provide more uniform plants for future experiments. Tarragon cuttings were placed in a 50/50 mix of sand and perlite in metal rooting trays, saturated, then placed on the misting bench at the UGA greenhouse on June 19, 1998. None of the cuttings produced roots. This suggests that tarragon stock would have to be grown from seed, introducing genetic variability into laboratory and field-scale experiments, and increasing the time required to produce the necessary plant material for phytoremediation.

4.4.3 Wetland Herbs

4.4.3.1 Pickleweed

When the selected pickleweed was transferred from the acclimation containers into the beakers used for the Run 2 of the initial experiments, new roots were observed in the experimental plants. However, because the condition of the above-ground portion of the majority of the plants was poor, the watering regime for the remaining plants not incorporated in the experiment was changed to a 5% NaCl solution made with DI water, with saturated, rather than moist soil conditions, as recommended by Dr. Ed Glenn and his staff. Saturated conditions were achieved by placing the plastic flats, containing 15 containers each, into plastic tubs, then filling the tubs with the saline solution to within approximately 3.0 cm of the soil surface. The remaining containerized pickleweed for future experiments are being maintained in a growth chamber. However, after being transferred to the growth chamber with the new watering regime, the condition of the plants did not appear to improve. The saline water in approximately one-third of the containers was replaced with freshwater again (saturated conditions) in an effort to increase the probability of some of the plants surviving. The plants in nonsaline water did not recover, but most of the remaining plants in the 5% NaCl solution stabilized after several weeks.

The sensitivity of pickleweed plants to transplanting and the uncertainty of success for germinating seeds at locations where on-site phytoremediation is needed will be significant factors that may influence field-scale performance of this species. In fact, future experiments of increased duration using this species may require significant modifications even to maintain control plants through the entire length of the experiment. The seed obtained at the initiation of these experiments is being maintained in a sealed plastic bag under ambient conditions in the laboratory. Because of the sensitivity of this species to transplant shock, the seed should be planted directly in containers to be used for testing (Ed Glenn, University of Arizona, pers. comm., 6/13/98). The impacts on viability of extended holding times, or attempted germination during periods other than the natural germination period are unknown.

4.4.3.2 Blue-hyssop

Blue-hyssop was the top performer with respect to mass of perchlorate depleted (6600 mg/kg). Additionally, this species did not exhibit any adverse responses to perchlorate or chloride in any treatments during the duration of the experiment. This species can be propagated vegetatively, and can cover large areas. Excellent field performance is predicted. However, blue-hyssop is a prime waterfowl food (Red Gidden, SMNWR, pers. comm., 7/28/98). Consequently, the potential threat to wildlife must be investigated.

4.4.3.3 Smartweed

This species can spread rapidly, by rooting at nodes, and can become established vegetatively in new areas. Although smartweed appears to be sensitive to chloride ions, it will take up perchlorate preferentially when chloride is present. The field performance of this species should be investigated.

4.4.3.4 Perennial Glasswort

Perennial glasswort is similar to pickleweed, except that the former grows rapidly via rhizomes, and is tolerant of transplanting. The smallest mass of perchlorate depleted in the presence of perennial glasswort was greater than the greatest mass of perchlorate depleted in the presence of pickleweed. The greatest mass of perchlorate depleted in the presence of perennial glasswort was the second largest mass depletion recorded during these experiments. These traits, and its tolerance of chloride, suggest that perennial glasswort will perform well in field trials.

4.4.4 Aquatic Herbs

4.4.4.1 Waterweed

This species has the potential for the greatest surface area of the aquatic species tested, and reproduces rapidly and vegetatively. It can grow throughout the water column in shallow water and in the upper zone of deeper water. Although no depletion of perchlorate occurred in the presence of this species during the experiment, different experimental conditions may mediate transformation of perchlorate by waterweed.

4.4.4.2 Parrot-feather

Parrot-feather also grows throughout the water column and reproduces rapidly and vegetatively. Its apparent ability to recover from chloride shock is an advantage for performance in the field, as is its adaptation to growth in both shallow and deeper water. Depletion of perchlorate in the presence of this species was observed in all treatments tested, supporting its range of tolerance and performance.

4.4.4.3 Fragrant White Water-lily

Water-lilies have large leaves and rhizomes (horizontal, underground stems), with robust, spongy roots that may be able to process contaminants in the sediment and water column. The rhizomes are extensive and should be able to be propagated readily by cross-sectional segments. The large biomass of this plant that extends throughout the water column should facilitate phytoremediation in aquatic systems. Dark stains in the sand of acclimation tanks suggest that this species produces root exudates even in the absence of perchlorate.

4.4.4. Duckmeat

This floating-leaved aquatic plant is small but spreads rapidly over the surface of the water via vegetative reproduction, particularly in the presence of nutrients. Duckmeat may be tolerant of chloride ions and perchlorate. Although no depletion occurred in the presence of Duckmeat during this experiment, other conditions may result in Duckmeat depleting perchlorate. For example, as individual plants die and decompose perchlorate may be transformed via Mechanism A (Figure 10).

5.0 SUMMARY AND RECOMMENDATIONS

Contamination of soil, surface water, and ground water with perchlorate represents a significant health risk in the United States. Remediation of contaminated sites using current technology is difficult and expensive. Microbial approaches for remediation of perchlorate have been successful at reducing perchlorate from 9000 ppm to approximately 500 ppm. However, perchlorate concentrations in contaminated ground water generally is in the range of 20 ppm, which may be below the range of efficiency for microbial applications.

The use of vascular plants for *phytoremediation* of soil and water contaminated with perchlorate is an emerging field of research. Initial experiments to evaluate the potential for phytoremediation of perchlorate by vascular plants included 13 species, and three concentrations of perchlorate (0.2, 2.0, and 20.0 ppm) in treatments with and without sand. The plants included four species of trees, one herbaceous upland species, four herbaceous wetland species, and four herbaceous aquatic species. The tree species were cabbage gum (*Eucalyptus amplifolia*), sweetgum (*Liquidambar styraciflua*), three age classes of eastern cottonwood (*Populus deltoides*), and black willow (*Salix nigra*). Tarragon (Artemisia dracunculus sativa) was the herbaceous species tested. Pickleweed (*Allenrolfea occidentalis*), blue-hyssop (*Bacopa caroliniana*), smartweed (*Polygonum punctatum*), and perennial glasswort (*Salicornia virginica*) were the four herbaceous wetland species tested. Waterweed (*Elodea canadensis*), parrot-feather (*Myriophyllum aquaticum*), fragrant white water-lily (*Myriophyllum aquaticum*), and duckmeat (*Spirodela polyrhiza*) were the four herbaceous aquatic species tested.

Results were favorable, but variable, suggesting that significant influences on depletion of perchlorate include: 1) plant species present, 2) concentration of perchlorate, 3) substrate (sand versus no-sand treatments), 4) the presence or absence of nutrients, 5) stage of plant maturity, and 6) the presence of chloride ions. For example, the presence of nutrients and other ions can inhibit depletion of perchlorate (*e.g.*, pickleweed, sweetgum); enhance depletion of perchlorate (*e.g.*, cabbage gum); or have no influence on depletion of perchlorate (*e.g.*, waterweed, duckmeat). Results from the modified experimental design, and the short duration of these experiments, supported the conclusion that depletion of perchlorate from solutions was not due to algal growth (primarily green algae) present in some treatments, or external microbes.

A preliminary sorption experiment with unwashed-sand and no plants revealed that 50-64% of perchlorate in solution became adsorbed to the sand, displacing chloride. Consequently, for treatments with unwashed-sand and plants in the subsequent three experiments, the free chloride ions in solution were available to be taken up by the plants. When perchlorate concentrations exceeded 2.0 ppm in unwashed-sand treatments, an option was available for plants to take up excess perchlorate, rather than chloride ions, from the solution.

Perchlorate was depleted from solution in the presence of all but two species tested (waterweed and duckmeat). The mass of perchlorate depleted (mg/kg wet plant weight) was classified into the following five general categories: 0 = no depletion; 1-99 = minimal depletion; 100-499 = moderate depletion; 500-999 = moderately high depletion; and >1000 = high depletion. None of the tree species tested, nor the herbaceous upland species tested were included in the highest category of performance. Wetland and aquatic plants included in the highest category were blue-hyssop, perennial glasswort, and parrot-feather. Results in the moderately-high category were obtained for one species of tree (cabbage gum), the herbaceous upland species (tarragon), and three of the four herbaceous wetland species evaluated (pickleweed, smartweed, and perennial glasswort).

Depletion of perchlorate was calculated as a pseudo-first-order kinetics reaction, with k

values (day-1) for sand treatments in the range of 0-0.22 for cabbage gum, sweetgum, rooted green-wood cuttings of cottonwood, black willow, pickleweed, smartweed, perennial glasswort, and fragrant white water-lily. Upper values for rooted mature-wood cuttings of cottonwood, blue-hyssop, and parrot-feather were 0.31, 0.34, and 0.41, respectively. The range for tarragon was 0.48-0.77. Tissues from plant organs (*e.g.*, roots, stems, leaves) were analyzed from selected samples, based on maximum drop in solution perchlorate concentration, for each of the 11 species for which perchlorate depletion was observed. Perchlorate, or transformation metabolites (chlorate, chlorite, and chloride) were observed in all tissue samples analyzed. Future work should include: 1) a quantitative analysis of each plant organ (*e.g.*, roots, stems, leaves), and 2) radiolabeled chloride to determine the amount and source of chloride contained within the plants, to evaluate the potential for chloride toxicity.

Characteristics of eastern cottonwood and black willow cuttings obtained from a site with perchlorate in the ground water, and incorporated into these experiments, suggested that fungal pathogens may be present in the donor plants on that site. Fungal pathogens, if present, may have influenced the performance of these plants in the experiment. Conversely, exposure of plants to perchlorate may create stresses that result in predispostion of the plant to infection by plant pathogens. Evaluation of these factors was not within the scope of these initial experiments, but should be addressed in future experiments. Another important aspect not evaluated in these short-term experiments was the potential environmental hazard to wildlife that may consume plants used for phytoremediation that contain high concentrations of perchlorate and transformation products. Future experiments of longer duration should provide more information regarding the degree to which perchlorate is accumulated in plant tissue, and any potential threat to wildlife.

The multitude of influential factors identified in these preliminary experiments and unexplored factors of concern necessitate additional research in the referenced areas to develop approaches for field application of vascular plants for phytoremediation. Specifically, future research should simulate specific site conditions to evaluate the role of factors such as nutrients and other ions (*e.g.*, chloride) that are present. Additionally, the influence of plant age and condition (*e.g.*, relationship of stress and predisposition to pathogens) on perchlorate uptake/transformation should be investigated. Experiments of extended duration also can evaluate long-term decline and recovery of plants, and identify the active period for enzymes in external transformation of perchlorate ("Type A" mechanisms).

Based on the results of these experiments and ecological knowledge of the species evaluated, the following species are recommended for future research for phytoremediation of perchlorate. The recommended plants are grouped by the type of phytoremediation for which they appear to be suited. Additional research using sweetgum, eastern cottonwood, and black willow is recommended for on-site and *in situ* phytoremediation of contaminated soils in uplands, including areas with shallow ground water accessible to plant roots, and if production of biomass for harvest is of interest. For on-site or *in situ* phytoremediation of contaminated areas that are saturated or inundated periodically, or for wetlands created for phytoremediation, additional research using blue-hyssop, smartweed, and perennial glasswort is recommended. Additional research using parrot-feather and fragrant white water-lily is recommended for on-site and *in situ* phytoremediation of contaminated waterbodies, or for ponds created artificially for phytoremediation of contaminated surface water or extracted ground water. Finally, extracts from tarragon may be useful for injection into mechanized flow-through reactors or plant systems where ground water is extracted, exposed to phytoremediation plants, then reinjected into the aquifer, or for similar flow-through systems for contaminated surface water. Related peer-reviewed publications and conference presentations are listed in Appendix C.

REFERENCES

Ataway, H. and Smith, M. (1993) Reduction of perchlorate by an anaerobic enrichment culture, *J Ind. Microbiol.,* 12, 408-412.

AWWARF (1998) Perchlorate Issue Group Presentations, *http://www.awwarf.com/newprojects/*

Bailey, L. H. and Bailey, E. Z. (1976) Hortus Third, A Concise Dictionary of Plants Cultivated in the United States and Canada. MacMillan Publishers, New York, NY. 1290 pp.

Brown, E. A. and Britton, K. O. (1986) *Botryosphaeria* diseases of apple and peach in the Southeastern United States. Plant Disease 70(5), 480-484.

California Department of Health (1998) Perchlorate in California drinking water. *http://www.dhs.cahwnet.gov/ps/dwem/chemicals/perch1/perchlindex.htm*

Glass, D. J. (1998) The 1998 United States market for Phytoremediation. *http://www.channel1.com/dglassassoc/infphytexec.htm*

Godfrey, R. K. (1988) Trees, Shrubs, and Woody Vines of Northern Florida and Adjacent Georgia and Alabama. The University of Georgia Press, Athens, Georgia. 734 pp.

Godfrey, R. K. and Wooten, J. W. (1979) Aquatic and Wetland Plants of the Southeastern United States: Monocotyledons. The University of Georgia Press, Athens, Georgia. 712 pp.

Godfrey, R. K. and Wooten, J. W. (1981) Aquatic and Wetland Plants of the Southeastern United States: Dicotyledons. The University of Georgia Press, Athens, Georgia. 933 pp.

Herman, D. C. and Frankenberger, W. T. Jr. (1998) Microbial-mediated reduction of perchlorate in groundwater, *J. Envrion. Qual*., 27, 750-754.

Jaeger, E. C. (1947) Desert Wild flowers, 4th Edition. Oxford University Press, London. 322 pp.

Phillips Laboratory (1997) Reclaimed ammonium perchlorate characterization for rocket propellants. *http://www.plk.af.mil*

Mabberley, D. J. (1997) The Plant Book, 2nd Edition. Cambridge University Press, Cambridge, MA. 858 pp.

Malmqvist, A., Welander, T. and Gunnarsson, L. (1991) Anaerobic growth of microorganisms with chlorate as an electron acceptor, *Appl. Environ. Microbiol*., 57, 2229 -2232.

Rikken, G. B., Kroon, A. G. M. and van Ginkel, C. G. (1996) Transformation of (per)chlorate into chloride by a newly isolated bacteriumn:reduction and disputation, *Appl. Microbiol. Biotechnol*., 45, 420-426.

Sinclair, W. A., Lyon, H. H., and Johnson, W. T. (1987) Diseases of Trees and Shrubs. Comstock Pub., Ithaca. N. Y. 574 pp.

Stanbury, J. B. and Wyngaarden, J. B. (1952) Effect of perchlorate on the human thyroid gland, Metabolism, 1, 533-539.

van Ginkel, C. G., Plugge, C. M. and Stroo, C. A. (1995) Reduction of perchlorate with various energy substrates and inocula under anaerobic conditions, *Chemosphere*, 4057-4066.

van Wijk, D. J. and Hutchinson, T. H. (1995) The ecotoxicity of chlorate to aquatic organisms: a critical review, *Ecotoxicol. Environ. Safety*, 32, 244-253.

van Ginkel, C. G., Rikken, G. B., Kroon, A. G. M. and Kengen, S. W. M. (1996) Purification and characterization of chlorite dismutase: a novel oxygen-generating enzyme, *Arch. Microbiol*., 166, 321-326.

Wallace, W., Beshear, S., Williams, D., Hospadar, S. and Owens, M. (1998) Perchlorate reduction by a mixed culture in an up-flow anaerobic fixed bed reactor, *J. Industril Microbiol. Biotechnol*., 20, 126-131.

Wunderlin, R. (1977) Guide to the Vascular Plants of Florida. University Press of Florida, Gainesville, FL. 806 pp.

APPENDICES

Appendix A.
Sources of Plant Species Evaluated and Pre-experimental Preparation

TREES:

Cabbage Gum (*Eucalyptus amplifolia* Naud.)

Only one source of *Eucalyptus* seedlings could be located at the time of these experiments. Dr. Don Rockwood, School of Forest Resources, at the University of Florida in Gainesville grew seedlings of three species, *Eucalyptus amplifolia*, *E. camaldulensis*, *E. grandis*, for potential production of mulch, fuel, pulp, and for experimental phytoremediation of heavy metals at municipal treatment sites. Unfortunately, only a limited number of one species *(E. amplifolia)* was available at the time of these experiments because all of the other seedlings had been planted on various research sites. These trees are evergreen in warmer areas, increasing the potential for "removal" of water through transpiration (Don Rockwood, pers. comm 6/98).

All of the *Eucalyptus* species originated in Australia; however, the seedlings being grown at the University of Florida were from many seed lots obtained from California. The seedlings of cabbage gum used in our experiment were from seed lot 4823. A limited number of seedlings of this species and the other two species may be available next summer; however, this is not a commercial source, and those seedlings are being grown for ongoing research at that facility.

The seedlings were approximately 35 cm tall and were germinated and grown under greenhouse conditions in 0.5 by 1.2 m plastic trays that hold 72 seedlings per tray. Seedlings were approximately 10 weeks old, and ready for transplanting to the field, or to larger containers at the initiation of the experiment. Seedlings were transported by automobile immediately prior to initiation of the experiment. The roots were washed by dipping the root mass repeatedly in a bucket filled with DI water, until all potting soil was removed.

Sweetgum *(Liquidambar styraciflua* L.)

The sweetgum seedlings were obtained from Rennerwood Nursery, Tennessee Colony, Texas, and were grown from seed collected locally. The seedlings were grown in 10 cm "root-makers" (custom-designed, inverted pyramid containers) and were approximately 60 cm tall. Twenty seedlings were shipped, bare-root, via UPS ground delivery for arrival on July 31, 1998, to be included in the final run of the experiment. The roots were washed as described above.

Eastern Cottonwood (*Populus deltoides* Bartr. ex Marsh.)

Mature-wood Cuttings:

Eastern cottonwood plant material was obtained as woody cuttings from mature trees growing on the Carswell AFB, Texas. The cottonwood cuttings reportedly were taken from branches of numerous two-year old trees that were planted on the AFB site and are now approximately 3.7 to 6 m tall (Glenn Rivers, USGS, pers. comm., 6/98). Consequently, the cottonwood cuttings are not identical genetically. The cuttings were enclosed in plastic bags and shipped overnight to the Athens, Georgia EPA facility in a plastic cooler containing block ice on June 16, 1998. The leaves had been removed from the cuttings prior to shipping, to reduce water loss from transpiration. The plant material was in excellent condition. Eighteen cuttings of similar diameter were selected to be rooted and used in the screening experiments. Diameters of the selected cottonwood cuttings ranged from approximately 1.0 to 2.5 cm. Minimum lengths of the selected cuttings were 40 cm.

The cuttings were placed in 3.5 L beakers with deionized (DI) water until June 19, 1998. On that date, each branch was cut (diagonal end cuts) to provide two 20 cm mature-wood segments

from each cottonwood branch. The newly-cut segments were labeled with consecutive numbers and "A" or "B", to designate lower and upper segments, respectively. These paired segments were selected randomly, for placement in either sand saturated with DI water or, seamless glass aquaria with a 5 cm depth of DI water. The water in the aquaria was aerated with a Reva Air 200 aquarium pump and 15 cm "aqua mist bar" to promote root growth. These plants were maintained in the laboratory, under artificial light (120 w Plant Gro N Show). By June 23, 1998, some of the cottonwood cuttings had produced new leaves approximately 2 cm in length.

The sand rooting bed and aerated water treatments were used because they coincided with the experimental treatments with and without sand. Roots produced in water are different, both structurally and physiologically, from roots produced in a solid substrate such as sand. Therefore, the preferred approach is to initiate the type of roots that will be acclimated to the treatment being used.

Unfortunately, only a small number (approximately 30%) of the similar-dimension cuttings selected for the two treatments produced roots after 19 days in the rooting treatments. Production of roots was most successful in the sand bed saturated with DI water, where 4 of 18 cuttings produced roots. One of the four cottonwood cuttings that produced roots in the sand bed had not produced leaves by July 14, 1998.

Results were less favorable in the other treatment (tank with aerated DI water), where only 1 of 18 cuttings produced roots. However, three cottonwood cuttings that did not produce roots, in addition to the one that had rooted, produced leaves in the aqueous rooting treatment during the same time span. On July 12, 1998, the unrooted cottonwood cuttings that had produced leaves in the aerated water were removed, and transfered to the sand bed. This was a final effort to induce root development in these mature-wood cuttings for the initial screening experiments.

The mature-wood cuttings that were not selected for these two rooting treatments and the green-wood cuttings of variable dimensions, were transferred to a second sand bed and placed under the mist system at the University of Georgia (UGA) greenhouse. The mist system routinely is used to root cuttings for commercial and research use. For the mature-wood cuttings of cottonwood under the UGA greenhouse mist system that were of similar-dimension to those selected for the screening experiment, only one of those cuttings had produced roots and leaves after 14 days. None of the remaining mature-wood cuttings of that size group had produced either roots or leaves during that same time period.

Green-wood Cuttings:

The sand bed under the mist system also contained a limited number of branch tip cuttings referred to as "green-wood" cuttings. These cuttings had not been selected to use in the Phase I screening experiment because there were not enough similar-sized green-wood cuttings to allow for natural attrition and still have enough cuttings for all treatments in the final experiment. In fact, only 8 of the 52 green-wood cuttings (15%) in the mist beds produced leaves and roots. A discussion of possible causes for the low percentage of root production in the cuttings is included in Section 4.5.

Four of the green-wood cuttings were similar in size and tissue components. A decision was made to include these four rooted cuttings in Run 3, for Treatments 1 and 6. This would provide a comparison of responses from tissue of two different age groups. Information of this nature would be useful in trying to predict field performance of cottonwood, and whether saplings might respond differently to perchlorate than older trees.

Seedlings:

No commercial sources for cottonwood seedlings were found in the vicinity of the Carswell AFB site. However a single source for cottonwood seedlings was located in Florida. The source was the same as the source for cabbage gum described above. The seedlings were approximately 15 cm tall, and were germinated in the same containers as the cabbage gum. The seed was collected from a number of natural populations throughout the southeastern United States. Seedlings from seed lot 4409 were selected because of the similar latitude and close proximity of that donor population (Tupelo, Mississippi) to the Carswell AFB site. After delivery of the seedlings to the laboratory, the roots were washed as described in cabbage gum, above, and the seedlings were incorporated into the final experiment, without acclimation.

Black willow (*Salix nigra* L.)

Willow cuttings from the Carswell AFB also were supplied for evaluation in these experiments. These cuttings reportedly were taken from a single tree approximately 4.6 to 6 m tall that is growing at the margin of the creek on the AFB site. Therefore, all of these cuttings have the same genetic composition. The bases of the willow cuttings ranged in diameter from approximately 0.7 to 1.0 cm. The minimum length of the cuttings were 30 cm. The willow cuttings were shipped with the cottonwood cuttings, as described above, with the same acclimation and rooting procedures, except that the eighteen comparable size willow cuttings were subdivided into two 15 cm segments for the experiment. At the time the initial leaves appeared on the cottonwood cuttings, new leaves approximately 2 mm also had appeared on the willow cuttings. This rooting response of the willow cuttings was similar to that of the cottonwood cuttings. A sufficient number of willow cuttings from each of the two rooting treatments produced roots and leaves so that this experiment could be conducted (9 of 18 cuttings rooted in sand and 11 of 18 cuttings rooted in aerated DI water). However, rooting success was low and insufficient for subsequent, large scale experiments.

UPLAND HERBS:

Tarragon *(Artemesia dracunculus* L.*)*

Thirty tarragon plants in 10 cm square plastic containers were obtained from Charmar Flower and Gift Shop in Athens, Georgia to be used in the first set of perchlorate/nutrient experiments. These perennial plants reportedly were germinated the preceding winter, were growing in typical potting soil, and had an extensive root system. Approximately half of the plants were removed from the 10 cm plastic containers, the roots were washed in the manner described for cabbage gum. The plants then were randomly assigned to either aerated DI water or sand for pre-treatment acclimation.

WETLAND HERBS:

Pickleweed (a.k.a. iodine bush, *Allenrolfea occidentalis* (Watson) Kuntze)

Three species in the genus *Allenrolfea* occur in the southwestern United States and Mexico. However, *Allenrolfea occidentalis* is the only species that is indicated to occur in the southwestern United States. The official common name assigned to this species is "pickleweed", although it also is referred to as iodine bush and picklebush. Technically the common name "iodine bush" refers only to the genus *Allenrolfea* (Phil Jenkins, University of Arizona, pers. comm., 6/11/98).

Pickleweed reportedly is fragile and does not transplant well (Ed Glenn, University of Arizona, James Henrickson, California State University, pers. comm., 6/98). Consequently, a source

for both plants and fresh seed was sought, so that plants would be available immediately for the initial experiments and seedlings could be grown for use in future experiments. Approximately 100 mature pickleweed plants and fresh seed with chaff were collected on June 28, 1998 by University of Arizona research personnel from a natural population at Rocky Point along the Sonoran Coast in Mexico. This is a research site for Dr. Ed Glenn, Environmental Research Laboratory, University of Arizona, Tucson, Arizona. The bare-root plants were wrapped in wet newspaper, and packed in a styrofoam-lined boxed. The fresh seed and chaff were in plastic ziplock bags, included in the box with the bare-root plants, and shipped to the Athens, Georgia EPA facility, via overnight Federal Express service. The plants arrived in excellent condition on June 30.

Thirty pickleweed plants of similar size and weight were transplanted into 10 cm square plastic containers in builder's sand. Prior to transplanting, the bare roots were rinsed in DI water, as described for cabbage gum, to remove any remaining soil. The containerized plants remained in the laboratory under grow lights to acclimate to conditions to be used in the screening experiment. The remaining small to medium-sized plants were transplanted as described for the plants selected for the second set of experiments.

Initially, all of the transplanted pickleweed remained in the laboratory, with soil moisture supplemented every other day using DI water to maintain saturation. Use of non-saline water during the acclimation period is recommended to promote the growth of new roots. Pickleweed reportedly turns bluish as the ion concentration in their tissue decreases to detrimental levels (Ed Glenn, University of Arizona, pers. comm., 6/98). This response did not occur. Instead, many of the plants became chlorotic, with flaccid apexes.

On July 8, 1998, the second set of experiments was initiated, with pickelweed included as one of the three test species. The seven most robust pickleweed were selected from the acclimation set of 30 plants, then randomly assigned to the seven treatments for pickleweed. When the selected pickleweed was transferred from the acclimation containers into the beakers used for the screening experiment, new roots (white in color) were observed in the experimental plants. However, the condition of the above-ground portion of the majority of the plants was poor.

Blue-hyssop *(Bacopa caroliniana*(Walt.) Robbins.)

Approximately 20 comparable-sized blue-hyssop plants were collected from a natural population located at the margin of the pondcypress wetland adjacent to Refuge Road 13, north of the Aucilla Tram Road, in the St. Marks National Wildlife Refuge (SMNWR) in Wakulla County, Florida (south of Tallahassee). The collected plants were wrapped in moistened paper towels and transported overnight to the EPA laboratory in Athens. After arrival, the plants were rinsed thoroughly with tap water, then DI water, and incorporated into the final set of experiments, without an acclimation period.

Smartweed *(Polygonum punctatum Ell.)*

Approximately 20 comparable-sized smartweed plants were collected from a natural population growing in the Headquarters Pond, 6.6 km (4.1mi.) south of Aucilla Tram Road 105, in SMNWR. The collected plants were placed in a plastic tub with water from the pond, and transported overnight to the EPA laboratory in Athens. After arrival, the plants were rinsed thoroughly with tap water, then DI water, and incorporated into the final set of experiments, without an acclimation period.

Perennial Glasswort *(Salicornia virginica L.)*

Approximately 30 comparable-sized perennial glasswort plants were collected from a natural population growing in a salt flat at the light house in the SMNWR. The sandy soil was removed from the roots by gently shaking the collected plants. Then the roots were wrapped in moist paper towels, and the plants transported overnight to the EPA laboratory in Athens. After arrival, the plants were rinsed in DI water, and incorporated into the final set of experiments, without an acclimation period.

AQUATIC HERBS:

Waterweed *(Elodea canadensis Rich.* in Michx.)

Waterweed was collected from an established population in the UGA Lake Herrick impoundment, located in close proximity to the Athens EPA laboratory. After collection, the plants were rinsed thoroughly with tap water, then DI water, and acclimated under plant growth lights in the laboratory where the experiments were conducted.

Parrot-feather (*Myriophyllum aquaticum* (Vell.) Verdc.)

Parrot-feather was obtained from an established population at Shaking Rock Park, in Lexington, Georgia, in the vicinity of the Athens EPA laboratory. After collection, the plants were cleaned and acclimated as described for waterweed.

Fragrant White Water-Lily (*Nymphaea odorata Ait.*)

Approximately 12 young specimens of fragrant white water-lily and 10 large, mature rhizome segments with roots and leaves were collected from a naturally established population growing at Mounds #1, in a ditch 2.9 km (1.8 mi.) north of Headquarters Pond, in SMNWR. The collected plants were placed in a plastic tub with water from the collection site, and transported overnight to the EPA laboratory in Athens. After arrival, the plants were rinsed thoroughly with tap water, then DI water. Eight of the young plants with similar-sized rhizomes, and similar leaf area were selected for incorporation into the final run of the experiment, without an acclimation period. The rhizomes of the larger plants, with leaves and roots, were cut into segments approximately 5 cm in length, and placed in a glass tank containing the same type of sand being used in the experiments, and DI water. Aeration was provided as described in section 3.1.1.3, above, and the tank was placed on a nursery bench in UGA Botany greenhouse #1, as a holding facility until the plants were needed for subsequent experiments.

Duckmeat (*Spirodela polyrhiza* (L.) Schleid.)

Duckmeat was obtained from an established EPA stock that was being maintained under plant growth lights in the laboratory. The plant material for this species used in the initial experiments was grown from 10 g of the laboratory stock population. Conditions for growth included placement in a shallow container filled with DI water, and with sufficient surface area to prevent overlap of the leaves. These plants were maintained under plant growth lights until initiation of the first experiment.

Appendix B.
Contents of Peters Professional All Purpose Plant Food*

Chemical Component	**Percent**
Total nitrogen (N)	20.00
Nitrate nitrogen	1.97
Urea nitrogen	18.03
Available phosphate (P205)	20.00
Soluble potash (K20)	20.00
Magnesium (Mg) (Total)	0.50
Magnesium (water soluble)	0.50
Boron (B)	0.02
Copper (Cu)	0.05
Chelated copper (Cu) 0.05	
Iron (Fe)	0.10
Chelated iron (Fe)	0.10
Manganese (Mn)	0.05
Chelated manganese (Mn)	0.05
Molybdenum (Mo)	0.0005
Zinc (Zn)	0.05
Chelated zinc (Zn)	0.05

* Derived from: potassium nitrate, urea, potassium phosphate, magnesium sulfate, boric acid, copper EDTA, iron EDTA, manganese EDTA, sodium molybdate, zinc EDTA.

Appendix C.
Related Peer-Reviewed Publications and Conference Presentations

Peer-ReviewedManuscripts Prepared for Publication:

1A. Phytotransformation of perchlorate and identification of metabolic products in *Myriophyllum aquaticum* - **International Journal of Phytoremediation** (S. Susarla, Bacchus, S. T., and McCutcheon, S. C.) Volume 1, pp. 97 - 107, 1999

1B. Phytoremediation of perchlorate by *Myriophyllum aquaticum* - **Soil and Groundwater Cleanup magazine** (S. Susarla, Bacchus, S. T., and McCutcheon, S. C.- abstracted by editors of *Inter. J. Phytoremediation*) Feb/Mar 1999

2. Uptake and transformation of perchlorate by vascular plants - **Environmental Science and Technology** (S. Susarla, Bacchus, S. T., and McCutcheon, S. C.) in press

3. Phytotransformation of perchlorate contaminated waters - **Water Research** (S. Susarla, Bacchus, S. T., Wolfe, N. L. and McCutcheon, S. C.) in press

4. Federal wetlands produce knights in shining armor for phytoremediation of perchlorate - **Science** (S. T. Bacchus, Susarla, S., Wolfe, N. L. and McCutcheon, S. C.) in review

Proposed Peer-Reviewed Publications and Intended Journal:

1. Uptake and transformation of perchlorate by trees: Environmental factors - **Environmental Toxicology and Chemistry** (Bacchus, S.T., Susarla, S., andMcCutcheon, S. C.)

2. Perchlorate and transformation products in vascular plant tissue: A mass balance approach - **Chemosphere** (S. Susarla, Bacchus, S. T., and McCutcheon, S. C.)

Conference Presentations:

1. Potential Species for phytoremediation of perchlorate- **Battelle Conference, San Diego, CA, April 1999.** (S. Susarla, Bacchus, S. T., and McCutcheon, S. C.)

2. Advantages of a multidisciplinary approach to on-site phytoremediation - **Georgia Water Resources Conference, Athens, GA, March 1999.** (Bacchus, S. T., Susarla S., and McCutcheon, S. C.)

3. Predicting field performance of herbaceous plants for phytoremediation of perchlorate - **ACS Conference, New Orleans, LA, August 1999.** (Bacchus, S. T., Susarla S., Wolfe, N. L., Harvey, G., and McCutcheon, S. C.)

4. Perchlorate uptake and transformation in aquatic plants - **15th Annual Contaminated Soils Conference, Amherst, MA, October 1999.** (S. Susarla, Bacchus, S. T., and McCutcheon, S. C.)

5. Uptake and transformation of perchlorate by trees - **15th Annual Contaminated Soils Conference, Amherst, MA, October 1999.** (Bacchus, S. T., Susarla, S., Harvey, G., Wolfe, N. L., and McCutcheon, S. C.)