PHYTOREMEDIATION OF PERCHLORATE BY NATIVE AND EXOTIC RIPARIAN PHREATOPHYTES OF THE LAS VEGAS WASH, NEVADA

by

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A thesis submitted in partial fulfillment of
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ABSTRACT

Phytoremediation of Perchlorate by Native and Exotic Riparian Phreatophytes of the Las Vegas Wash, Nevada

by

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The potential to use native (*Salix exigua*) and exotic (*Tamarix ramosissima*) phreatophytes to remediate perchlorate from arid riparian environments was investigated by conducting a hydroponic greenhouse experiment. *Salix exigua* and *Tamarix ramosissima* exposed to ammonium perchlorate at concentrations of 10 mg L^{-1} and 100 mg L^{-1} removed 15 to 22% perchlorate mass from hydroponic solution, with 55 to 64% of removal being taken up into plant tissue. Total perchlorate taken up by plants or removed from solution was not significantly different between species on a mass or oven dry plant weight concentration basis. Significant differences in tissue specific uptake, however, were detected, with *Salix exigua* accumulating 78 to 87% of perchlorate in the leaf and *Tamarix ramosissima* exuding 84 to 87% of uptake onto leaf surfaces. Burning leaf tissue resulted in no detectable perchlorate in ash.

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CHAPTER 1

INTRODUCTION

Perchlorate in Southern Nevada

Detectable concentrations of perchlorate (ClO_4^-) naturally occur in surface water and groundwater in the western United States (U.S.) (Snyder et al. 2005, Plummer et al. 2006) which may be a result of atmospheric processes (Dasgupta et al. 2005). However, several major waterways in the western U.S. are highly contaminated with anthropogenic sources of perchlorate (U.S. Environmental Protection Agency 2006). Contamination in the lower Colorado River has been traced to Southern Nevada, where ammonium perchlorate and other perchlorate salts were historically manufactured near Henderson, Nevada. Perchlorate was manufactured at an industrial facility most recently owned by Kerr-McGee Corporation but now operated by Tronox Incorporated and at another nearby manufacturing facility, the Pacific Electrochemical Production Company owned by American Pacific Corporation. Concern over perchlorate in Southern Nevada began in 1997 when elevated concentrations were detected in surface waters of the Las Vegas Wash, Clark County, Nevada. The source of the perchlorate was determined to be a contaminated groundwater plume originating from the former manufacturing facilities (U.S. Environmental Protection Agency 2006). Because the Las Vegas Wash is the primary conduit for draining Las Vegas Valley's 4,150 km^2 hydrographic basin, which

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captures shallow groundwater, storm water, urban runoff, and treated wastewater flows, contaminants within the watershed are also transported through this channel.

The Las Vegas Wash discharges water from the Las Vegas Valley into Lake Mead, a reservoir on the lower Colorado River. Las Vegas is dependent on Lake Mead and the lower Colorado River for drinking water, as are millions of downstream users in California and Arizona. Colorado River water is also used for irrigating crops in southern California's Imperial Valley, one of the largest agricultural areas in the country, and other agricultural districts in California, Arizona, and Mexico. Perchlorate contamination from the Las Vegas Wash has the potential to affect human health through the consumption of perchlorate laden drinking water or agricultural food stuffs, and it may also affect aquatic dependent organisms. For example, Brechner et al. (2000) observed elevated thyroid stimulating hormone (TSH) levels in newborns in a city supplied by lower Colorado River water. In another study, lettuce irrigated with lower Colorado River water was shown to have perchlorate, although the amounts were negligible (Sanchez et al. 2005). Further evaluation of the toxicological relevance of perchlorate at trace levels and treatment alternatives should be conducted (Logan 2001; Snyder et al. 2003).

Protected Species, Riparian Habitat Restoration, and Perchlorate Phytoremediation

The lower Colorado River (i.e., below Glen Canyon Dam) provides habitat for several threatened and endangered species of fish and wildlife, including the federally listed razorback sucker (*Xyrauchen texanus*) and bonytail chub (*Gila elegans*) (Mueller

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and Marsh 2002). The U.S. Fish and Wildlife Service has in recent years become particularly interested in learning more about the relevance of perchlorate in the environment and the potential effects of this contaminant on trust resources like the razorback sucker and bonytail (Tuttle and Orsak 2002). The Department of Defense (DoD) is also interested in perchlorate in the environment because of the various DoD facilities that have unintentionally contaminated surface water and groundwater with perchlorate (U.S. Army Corp of Engineers 2004; Smith et al. 2001). To determine if perchlorate affects the thyroid and reproductive systems of fish, the DoD funded studies in Lake Mead to determine the toxicological impact of perchlorate on carp (*Cyprinus carpio*). Carp were chosen for the study as a substitute for the endangered razorback sucker. Perchlorate salts are highly water-soluble and they do not biomagnify in organisms. Rather, perchlorate contaminated waters affect the growth and reproductive functions of animals only while they live in these waters. This could have obvious detrimental impacts for the continued existence of rare species living in highly contaminated water. Other aquatic and riparian dependent taxa living in perchlorate-contaminated waterways may also be affected.

Interestingly, activities being conducted to promote the recovery of some riparian dependent species may alter the presence and distribution of perchlorate in the environment. Specifically, resource management decisions that promote restoring habitat for the federally endangered southwestern willow flycatcher (*Empidonax traillii extimus*) could have unknown consequences for the environmental cycling of perchlorate. Urbansky et al. (2000) have shown, for example, that the non-native *Tamarix ramosissima* phytoaccumulates perchlorate and therefore activities to remove this

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vegetation for habitat restoration reasons may be consequential. Anthropogenic disturbances, including river management practices, have had a profound impact on native riparian plant taxa, which are important habitats for the southwestern willow flycatcher (Sogge et al. 1997). Habitat degradation, partially due to the introduction of exotic species in the southwestern U.S., is regarded as the primary cause for southwestern willow flycatcher decline (U.S. Fish and Wildlife Service 1993). Habitat restoration initiatives that remove non-native *Tamarix* may potentially alter phytoremediation dynamics in these systems.

In many parts of the country, the non-native invasive *Tamarix* has displaced much of the native riparian vegetation (Graf 1978; Turner and Karpiscak 1980; Brock 1994; DiTomaso 1998) and reduced habitat quality for riparian-dependent vertebrate taxa. Along the lower Colorado River riparian corridor, *Tamarix* spp. have contributed to the decline of many native woody plants, including Fremont cottonwoods (*Populus fremontii*) and willows (*Salix* spp.) (Busch and Smith 1995) partially due to its superior drought and salinity tolerance (Cleverly et al. 1997; Glenn et al. 1998; Vandersande et al. 2001). *Tamarix* spp. has also been documented to increases fire frequency in these riparian areas (Busch 1995), which further promotes *Tamarix* spp. infestation by increasing sprout regeneration (Busch and Smith 1993).

Tamarix ramosissima is a facultative phreatophyte (Smith et al. 1998) unlike genera of *Populus* and *Salix*, which are considered obligate phreatophytes. Facultative phreatophytes are able to extract water at or near the phreatic zone but they can also tolerate periods of ground water depression (i.e., soil drying). The latter trait offers *Tamarix* a competitive advantage, particularly considering the changes that have occurred

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in many river systems in the western U.S. as a result of river management practices. Consequently, it has been suggested that re-establishing native vegetation in these systems will require restoring natural hydrodynamics (Levine and Stromberg 2001; Stromberg 2001; Tallent-Halsell and Walker 2002). There are, however, a variety of programs already initiated along the Colorado River corridor to enhance native *Salix* and *Populus* habitats that do not necessarily use hydrodynamic alterations as a management tool.

Along the Las Vegas Wash, a variety of improvement initiatives have been conducted in the past several years with the intent of restoring ecological functions that have been diminished. Historically, the Las Vegas Wash was a dry ephemeral channel, which carried periodic high velocity storm flows, but because of the development of Las Vegas's water resources the Las Vegas Wash has been converted into a perennial stream. During the conversion process, the invasive *Tamarix* began to dominate and degrade the floodplain. Perennial flows and several major flood events further degraded this system by eroding much of the original floodplain of the Las Vegas Wash. A series of stabilization structures are being constructed in and along the banks of the Las Vegas Wash to protect it from further erosion. These activities improve the hydrology of the channel so that it can support native plants. Although improvement initiatives require removing *Tamarix* and replacing it with native species, *Tamarix* has been shown to accumulate perchlorate (Urbansky et al. 2000) and therefore this vegetation community shift may have unintended consequences for the cycling of perchlorate near the Las Vegas Wash.

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Objectives and Hypotheses

The purpose of the present study was to evaluate the potential for *Salix exigua* (herein referred to as *Salix*) and *Tamarix ramosissima* (herein referred to as *Tamarix*) to uptake perchlorate. *Tamarix* was chosen for this study because it is the most dominant woody species found along the Las Vegas Wash and *Salix* was chosen because restoration initiatives are replacing *Tamarix* with *Salix*. Since there may be differences in uptake capacity by these plants and as restoration initiatives continue along the Las Vegas Wash, the dynamics of perchlorate in the environment may be altered. A short duration greenhouse experiment was designed to determine the fate of perchlorate in plants that were exposed to various levels of perchlorate from hydroponic solution. Although the general objective for this study was to evaluate the potential for using phytoremediation as a treatment alternative along the Las Vegas Wash, the specific objectives of the experiment were:

Objective 1 To describe the fate of perchlorate in the roots, stems, and leaves of *Tamarix* and *Salix* growing in hydroponic solution at various perchlorate concentrations;

Objective 2 To determine species specific perchlorate accumulation and solution removal dynamics;

Objective 3 To determine whether perchlorate is exuded onto the leaf surfaces of the two species while growing in hydroponic solution;

Objective 4 To describe the potential for perchlorate cycling back into the environment as a result of leaf fall; and

Objective 5 To determine if burning perchlorate laden leaf material results in a source

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of perchlorate that can cycle back into the environment.

In order to achieve these objectives, the following hypotheses were evaluated:

Hypothesis 1 Perchlorate phytoaccumulates in *Tamarix* and *Salix* roots, stems, and leaves;

Hypothesis 2 Plant tissue perchlorate concentrations are greatest in the leaf followed by the stems and the roots;

Hypothesis 3 *Tamarix* phytoaccumulates more perchlorate than *Salix* under similar conditions;

Hypothesis 4 *Tamarix* and *Salix* phytoaccumulate perchlorate at environmentally relevant levels;

Hypothesis 5 *Tamarix* and *Salix*, while growing in hydroponic solution, do not exude perchlorate salts onto their leaves;

Hypothesis 6 *Tamarix* and *Salix* leaf fall are significant pathways for cycling perchlorate back into the environment; and

Hypothesis 7 Leaf ash from these two species is not a source of perchlorate to the environment.

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CHAPTER 2

BACKGROUND

Perchlorate in the Environment

The perchlorate ion (ClO_4^-) is a negatively charged inorganic ion that is often combined with other elements to form salts, such as sodium perchlorate ($NaClO_4$) and ammonium perchlorate (NH_4ClO_4) (Espenson 2000). Perchlorate adsorbs poorly to mineral surfaces and perchlorate salts dissolve readily in water. Perchlorate is generally a poor oxidizer at low temperatures. However, at high temperatures perchlorate can be a strong oxidizing agent, which is why ammonium perchlorate has been used extensively in both the defense and aerospace industry because of its application as an oxidizer for solid rocket propellants. It has also been used for fireworks, road flares, air-bag inflation systems, lubricating oils, and nuclear reactors.

Perchlorate contamination in aquatic environments is a concern because it interferes with iodine uptake in the thyroid gland (Wolff 1998). Perchlorate competitively inhibits the transport of iodine into the thyroid by binding with the sodium iodide symporter (NIS) (Josefsson et al. 2006). The NIS protein molecule is a mediated pathway for iodine to enter into the thyroid and because perchlorate is ionically similar to iodine, NIS often uptakes perchlorate instead of iodine. The thyroid gland is part of the endocrine system and is therefore important for regulating metabolism in adults and is critical for regulating growth in infants and children. Iodine is an important component of the

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thyroid hormones triiodothyronine (T_3) and thyroxine (T_4), and they are important for regulating growth, cell separation, and lipid, protein, and carbohydrate metabolism (Capen and Martin 1989, Lehninger et al. 1997). Consequently, if perchlorate is taken into the thyroid instead of iodine, intrathyroidal iodide deficiency may occur resulting in a reduction in the synthesis of T_3 and T_4.

In the late 1990s perchlorate was found in surface water from many parts of the U.S. as a result of a new analytical method that was able to identify concentrations of perchlorate lower than the existing laboratory reporting limit. The scientific and regulatory community has over the past several years been trying to understand the relevancy of perchlorate exposure in humans. Because of the potential health concern associated with perchlorate that had been detected in waters throughout the U.S., the Environmental Protection Agency (EPA) listed perchlorate on the Contaminant Candidate List and under the Unregulated Contaminants Monitoring Rule (UCMR) (Browner 1999). Under the UCMR, perchlorate monitoring is required to be conducted at a minimum detection level of 4 $\mu g\ L^{-1}$ at all drinking water facilities. In 1999, the EPA released the Interim Assessment Guidance for Perchlorate (U.S. Environmental Protection Agency 1999) which recommended that EPA risk assessors and risk managers continue to use the provisional reference dose (RfD) range of 0.0001-0.0005 $mg\ kg^{-1}\ day^{-1}$ while the toxicological relevance of perchlorate in the environment was being evaluated by the scientific community. Based on a standard default body weight of 70 kg and a water consumption rate of 2 $L\ day^{-1}$, the resulting action level would be 4-18 $\mu g\ L^{-1}$. Although RfD's are not drinking water standards, they are scientifically valid estimates of daily exposure that are not expected to result in adverse human health effects. In

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Nevada, consequently, the Nevada Division of Environmental Protection (NDEP) established a provisional action level of 18 $\mu g\ L^{-1}$ to protect public health and safety and the environment. The action level designation is the concentration at which corrective remedial action may be required of an owner or operator of a facility that has released perchlorate into the environment.

In 2005, the National Research Council (NRC) of the National Academies assessed the state of the science for perchlorate health implications (NRC 2005). This involved a comprehensive evaluation of adverse health effects in both humans and laboratory animals. Based on the NRC review, the EPA established an official RfD for perchlorate of 0.0007 $mg\ kg^{-1}\ day^{-1}$ or 25 $\mu g\ L^{-1}$. From the RfD, the EPA can develop a national drinking water standard (i.e., a maximum contaminant level) for perchlorate, however, this process can take several years.

Uptake and Degradation of Perchlorate by Plants

Bioremediation is the process by which contaminated surface water, groundwater, or soils are cleaned through a biologically mediated pathway. The remediation of contaminants can occur at multiple scales and by various mechanisms, which may include microbial or whole-plant mediated processes. In situ plant mediated remediation of contaminated sites is typically known as phytoremediation. Phytoremediation is an emerging technique that has been used to treat various contaminants including metals, pesticides, solvents, explosives, crude oil, polycyclic aromatic hydrocarbons, and landfill leachates (Terry and Banuelos 2000). Phytoremediation can be divided into several subcategories including phytoaccumulation, phytodegradation, phytovolatilization, and

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rhizodegradation, with each category described by its remediation mechanism. For example, phytoaccumulation is the uptake and translocation of a contaminant into plant tissue and phytodegradation is the uptake, translocation, and degradation of a contaminant to a non-contaminant compound within the plant. Phytoremediation techniques are a preferred alternative to conventional remediation technologies because they are an aesthetically pleasing passive alternative that is typically cost effective (Susarla et al. 2002). For perchlorate, three mechanisms have been reported to be important components of phytoremediation; phytoaccumulation, phytodegradation, and rhizodegradation (Nzengung et al. 1999; Aken and Schnoor 2002). The present study proposes to evaluate the importance of perchlorate phytoaccumulation in two woody plant species.

A multitude of studies have shown that plants uptake and degrade perchlorate. Nzengung et al. (1999) and Nzengung and Wang (2000) documented perchlorate uptake in willows (*Salix nigra* and *S. caroliniana*). Nzengung et al. (1999) documented the degradation of perchlorate to chloride, which was facilitated by rhizosphere associated microorganisms. They also documented that high nitrate concentrations and the source of nitrogen in the nutrient solution interfered with the degradation of perchlorate. Susarla et al. (1999) observed that the perennial herbaceous watermilfoil (*Myriophyllum aquaticum*) accumulated 1200 mg kg^{-1} of perchlorate on a fresh weight basis. Watermilfoil is an introduced species and it is closely related to the Eurasian watermilfoil (*Myriophyllym spicatum*), a noxious weed in Nevada. Susarla et al. (2000) also observed that perchlorate can be taken up by the native forb dotted smartweed (*Polygonum punctatum*), the perennial salt tolerant shrub iodinebush (*Allenrolfea occidentalis*), the

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sweetgum tree (*Liquidambar styraciflua*), the herbaceous American white waterlily (*Nymphaea odorata*), and the black willow tree (*S. nigra*).

Tobacco plants (*Nicotianum tabacum*) were shown to uptake perchlorate and off-the-shelf tobacco products were shown to have up to 60 mg kg^{-1} of perchlorate on a fresh weight basis (Ellington et al. 2001). Other consumable products, including garden cucumber (*Cucumis sativus*), garden lettuce (*Lactuca sativa*), and soybean (*Glycine max*) were shown to uptake perchlorate at maximum concentrations of 750 mg kg^{-1}, 41 mg kg^{-1}, and 18 mg kg^{-1}, respectively (Yu et al. 2004). Store bought garden lettuce and spinach (*Spinacia oleracea*) were shown to have a range of 0.6 to 6.4 μg kg^{-1} of perchlorate on a wet weight basis (Seyfferth and Parker 2006).

Aken and Schnoor (2002) used radio-labeled $^{36}ClO_4^-$ and observed perchlorate uptake and degradation in poplar trees (*Populus deltoide* x *nigra*). In an assessment of receptors on the Longhorn Army Ammunition Plant in Karnack, Texas, Smith et al. (2001) detected perchlorate in bulrush (*Scirpus* sp.), crabgrass (*Digitaria* sp.), cupgrass (*Erichloa* sp.), and goldenrod (*Solidago* sp.) at maximum concentrations of 9.5 mg kg^{-1}, 5,557 mg kg^{-1}, 1,060 mg kg^{-1}, and 1,030 mg kg^{-1}, respectively (dry weight). In a similar study along the Las Vegas Wash, Smith et al. (2004) detected mean perchlorate concentrations of 645 mg kg^{-1}, 521 mg kg^{-1}, 429 mg kg^{-1} (wet weight) in plants that were classified as terrestrial broadleaf, seeding bush, and aquatic broadleaf, respectively. Also along the Las Vegas Wash, Urbansky et al. (2000) detected perchlorate in dormant stems of *Tamarix*. They found 300 mg kg^{-1} of perchlorate in submerged *Tamarix* stems and 5-6 mg kg^{-1} of perchlorate in dry stems from the same plant. The mechanisms by which *Tamarix* takes up perchlorate and the rate it takes it up were not investigated. In addition,

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it is not clear where perchlorate may be stored in the plant since leaves and roots were not sampled. There may be selective partitioning of perchlorate in the roots, stems, or leaves. This may be important since *Tamarix* is a deciduous plant and consequently leaf fall may contribute significantly to the cycling of perchlorate in the environment, and the potential movement of perchlorate from groundwater to surface water. Tan et al. (2004) documented uptake of perchlorate by various species including ash, hackberry, and willow, which had maximum dry weight perchlorate concentrations of 17 mg kg^{-1}, 48.2 mg kg^{-1}, 24.3 mg kg^{-1}, respectively, and they found that leaf concentrations were generally greater than other tissue concentrations. They found substantially higher concentrations of perchlorate in china-berry and mulberry leaves than fruit.

History and Importance of the Las Vegas Wash and Significance for Perchlorate Phytoremediation

Wetlands and the riparian zones adjoining wetlands provide many benefits, including habitat for fish and wildlife, flood and erosion control, water filtration, siltation control, and opportunity for recreation, education, and research. Over half of the wetland areas in the U.S. were lost between the 1700s and the mid 1980s. It has only been within the last few decades that our understanding and appreciation of wetland functions, values, and services has greatly increased. A great deal of our current knowledge on wetland function and value to wildlife has been identified through rigorous investigations by federal, state, and local agencies, academic institutions, and the private sector (see e.g., Carothers et al. 1974; Stevens et al. 1977; Knopf 1985; Knopf et al. 1988). Although riparian zones make up less than 1% (Knopf et al. 1988) of the total land area in the

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southwestern U.S., their importance to wildlife is invaluable. Riparian zones support 75%-80% of the wildlife species in the region (Gillis 1991). In addition, 80% of the breeding bird population and 50% of the protected migratory bird population rely on riparian zones (Wharton et al. 1982).

Within Clark County, Nevada, lowland riparian zones (i.e., less than 1200 meters) are found along the Virgin, Muddy, and Colorado Rivers, and along the Las Vegas Wash. These unique desert riparian ecosystems make up a small percentage of the total land area within the county but provide essential cover, water, food, migration, and breeding sites for a variety of wildlife species in this otherwise arid desert environment (Bradley and Niles 1973, U.S. Fish and Wildlife Service 1995, BIO/WEST 2001, Mueller and Marsh 2002, Las Vegas Wash Coordination Committee 2003). For example, up to 70% of Southern Nevada's avifauna is found in these riparian communities (Bradley and Deacon 1965).

The Las Vegas Wash serves as a locally unique environment in which a variety of organisms not commonly found in the arid Mojave Desert thrive. The Las Vegas Wash exists in its present state because of the rapid urbanization of Las Vegas Valley following the construction of the railroad between Salt Lake City and Los Angeles in 1905. Prior to urbanization, much of the Las Vegas Wash was an ephemeral desert drainage channel characterized by typical wash scrub vegetation. Although the Las Vegas Wash has an estimated annual mean flow of 275 cubic feet per second (cfs), as measured by the U.S. Geological Survey stream gage at Pabco Road for water year 2004, which is greater than that of small rivers flowing into the lower Colorado River basin such as the Virgin River (113 cfs for water year 2004 at Littlefield, AZ) and the Muddy River (8.8 cfs for water

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year 2004 at Overton, NV) (U.S. Geological Survey 2004), the term "wash," which is defined as a "dry bed of an intermittent stream" is reminiscent of the more pre-settlement era. Although parts of the Las Vegas Wash were typical of a "dry bed of an intermittent stream," there were perennial flows from Las Vegas Springs, which created the Las Vegas Creek in the central part of the Las Vegas Valley.

The development of the Las Vegas Valley in the 1930s, 1940s, and 1950s resulted in the input of surface water flows into the Las Vegas Wash. Annual mean flow by 1962 was 16.9 cfs (U.S. Geological Survey 2004) but as the population of Las Vegas grew, the discharge of treated wastewater into the Las Vegas Wash increased, which resulted in increased total flow (Figure 1). The addition of this new water supply to the Las Vegas Wash resulted in permanent surface water flows and elevated groundwater levels, which caused a transition from xeric and mesic plant communities to more hydric plant communities. The Las Vegas Wash slowly started to transform from a desert wash to a desert riparian ecosystem. During this change, pioneering plants of which many are non-native became dominant. In the 1960s, the amount of water that was flowing in the Las Vegas Wash reached a critical point in which wetland vegetation could no longer use all of the channel's flow. Compounded by periodic and destructive flood events, the delicate floodplain started to erode, resulting in habitat, topographic, and water quality alterations.

The importance of the Las Vegas Wash to the Las Vegas watershed was recognized early on, and a variety of groups were formed and plans developed to address Las Vegas Wash related issues. The most recent attempt by the Las Vegas community to manage this resource began in 1998 with the formation of the Las Vegas Wash Coordination Committee, a 29-member group of federal, state, and local agencies, citizen groups,

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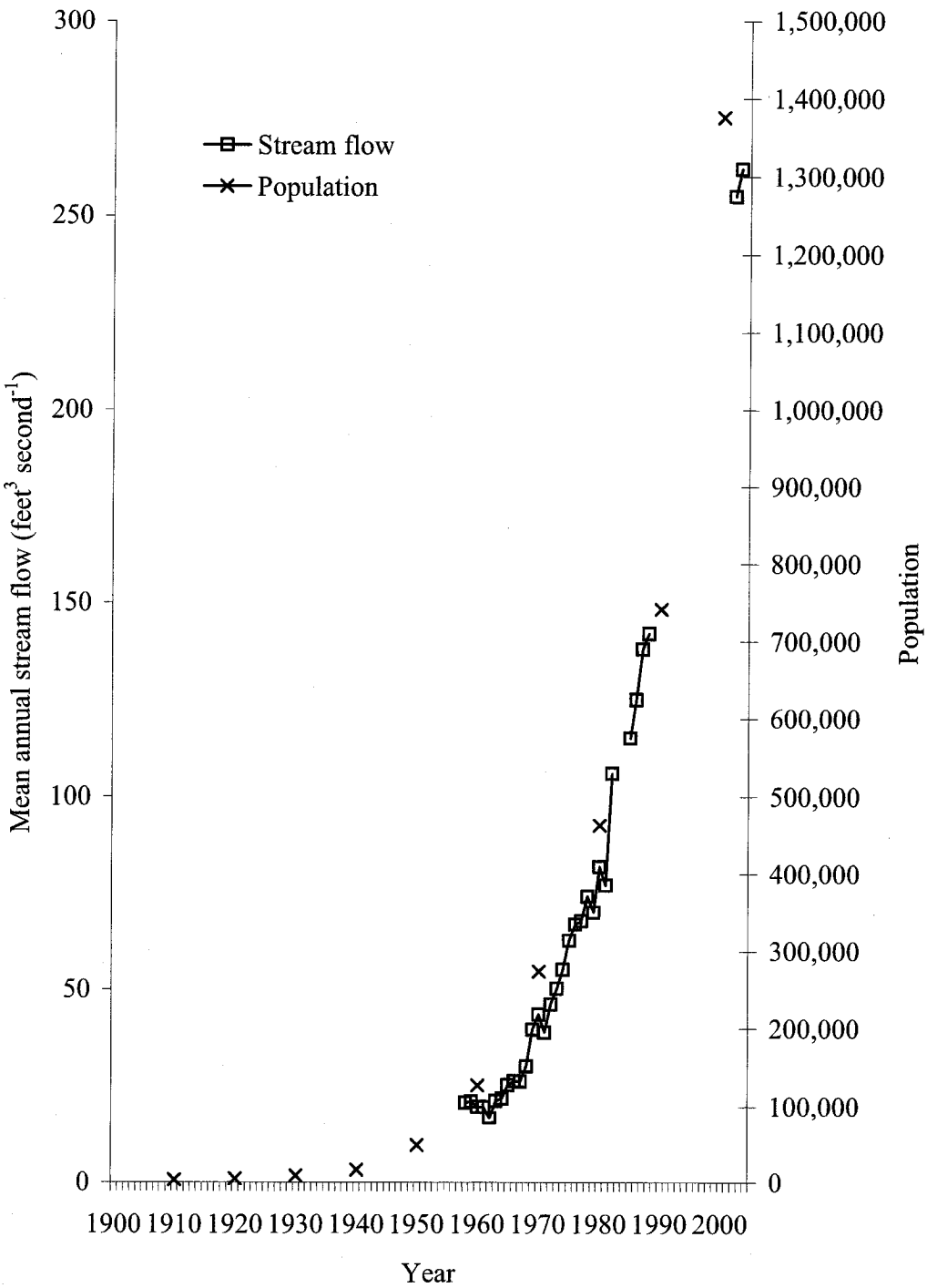
Figure 1. Comparison of population size and Las Vegas Wash discharge.



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stakeholders, and private businesses and organizations. By 2000 the Las Vegas Wash Coordination Committee had developed the Las Vegas Wash Comprehensive Adaptive Management Plan (CAMP) that outlined 44 recommendations that would need to be accomplished to help manage the Las Vegas Wash.

The CAMP outlined that a single agency should be appointed to coordinate and oversee work being conducted in the Las Vegas Wash and the Southern Nevada Water Authority (SNWA) was ultimately appointed as this agency. The SNWA provides coordination and oversight services under a framework directed and supervised by the Las Vegas Wash Management Advisory Committee (i.e., appointees from Clark County, Clark County Water Reclamation District, Clark County Regional Flood Control District, City of Henderson, City of Las Vegas, City of North Las Vegas, and SNWA) and several study teams.

A variety of erosion control activities are currently being implemented along the Wash as part of a comprehensive stabilization strategy aimed at returning lost ecological functions to this system. These activities include the construction of grade control and stream bank protection structures. After erosion control facilities have been constructed, SNWA engages in an equally comprehensive program to revegetate surrounding areas with native vegetation. The general goals for revegetation activities along the Wash are to develop ecologically functioning wetland, riparian, and upland areas that are self-sustaining in the long-term. Revegetation activities along the Wash do not attempt to restore the pre-settlement desert vegetation nor the post-settlement non-native vegetation; rather, these activities attempt to create similar vegetative conditions found along many of the riparian drainages of the lower Colorado River basin.

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Native plants used for revegetation along the Wash include Fremont cottonwood (*Populus fremontii*), Goodding's willow (*Salix gooddingii*), sandbar willow (*S. exigua*), western honey mesquite (*Prosopis glandulosa* var. *torreyana*), screwbean mesquite (*Prosopis pubescens*), Emory's baccharis (*Baccharis emoryi*), wolfberry (*Lycium andersonii*), saltbush (*Atriplex* spp.), and bulrush (*Schoenoplectus* spp.). These species are planted in areas of suitable hydrologic and edaphic conditions. Revegetation sites along the Wash provide suitable environmental conditions for these riparian species as well as for other more desert adapted species like creosote bush (*Larrea tridentata*) and bursage (*Ambrosia dumosa*). Revegetation sites are generally designed to maximize native vegetative coverage, while also providing for physiognomic features that mimic native riparian conditions.

Currently, *Tamarix* is the dominant woody plant in the Las Vegas Wash and there are programs in place to eradicate it and replace it with native vegetation (Bickmore 2003; Kloeppel et al. 2006). Similar eradication programs are currently underway in the lower Colorado River drainage (Deuser et al. 1998). Urbansky et al. (2000), however, have suggested that *Tamarix* could be used for phytoremediation and that eradication would result in unintended consequences. Further, depending on the eradication technique, downed plant material that remains on site may be a leachable source of perchlorate. Pomeroy et al. (2000), for example, documented 70% leaf pack decomposition over 142 days for leaves of *Tamarix chinensis* which suggests that accumulated perchlorate may be released back into the environment when invertebrates and microbes decompose leaf material. Since *Tamarix* is deciduous, leaf fall from live stands may also be a source of perchlorate cycling. Additionally, *Tamarix* is physiologically capable of exuding salts

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onto its leaves, and if it is growing in a perchlorate contaminated waterway, these salts may contain perchlorate. Exuded salts may be another contributing source of perchlorate.

Although *Tamarix* is a desirable plant for phytoremediation because it transpires large volumes of water (Glenn et al. 1998; Nagler et al. 2003) other species may be equally as desirable. For example, Nzengung and Wang (2000) have reported that willows (*Salix* spp.) uptake perchlorate and could be used for phytoremediation. It is not known, however, if *Salix exigua* (sandbar willow), a willow native to the southwestern U.S. and a species typically used for habitat restoration accumulates perchlorate. *Tamarix* eradication and subsequent native revegetation along the lower Colorado River and the Las Vegas Wash is a preferred resource management goal, because it will increase habitat for many riparian dependent taxa and restore ecological function to these systems. If *Salix* is found to accumulate perchlorate equal to or more than *Tamarix*, revegetation goals to improve wildlife habitat may also have the unintended consequence of helping to meet water quality goals for many lower Colorado River users.

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CHAPTER 3

MATERIALS AND METHODS

In order to test the hypotheses stated above, a short duration greenhouse experiment was conducted under natural lighting. Information collected from this study significantly expands the current knowledge of perchlorate phytoaccumulation in the exotic phreatophyte *Tamarix*, and the native phreatophyte *Salix*, a species typically planted at ecological restoration sites. Results from this experiment may help align water quality and habitat restoration goals for many western U.S. resource managers.

Greenhouse and Hydroponic Tank Setup

The hydroponic study was conducted at the University of Nevada, Las Vegas greenhouse facility located on the university campus. The greenhouse facility consists of three temperature controlled, glass enclosed growing bays used to insulate plants from extreme Las Vegas weather. The west bay, where this study was conducted, is further protected from the external environment by a series of moderately permeable shade cloths on its roof. Temperature (°C), relative humidity (%), and carbon dioxide (μmol mol^{-1}) were recorded with sensors installed on the inside of the greenhouse. Data were later downloaded to a computer. A reverse osmosis water treatment system was used as a water supply during the experiment.

Thirty-six custom made hydroponic tanks that were fabricated by an earlier investigator (Grant 2003) were used in this study. Although the number of tanks that

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were used to propagate plants at the beginning of the study (36) was greater than the number desired for experimental treatment (24), it was important to initially plant more tanks then needed to account for potential die off during the establishment period. The original tank design was modified slightly to maintain clean growing conditions (see below). Tank walls were prepared from cut sections of white polyvinyl chloride (PVC) piping approximately 28.5 cm in length and an inside diameter of approximately 20 cm. The PVC piping was then tilted vertically and attached at its base to 30 cm by 30 cm square sections of white sheet PVC. Average tank volume was approximately 8 L. Contact points for the piping and sheet PVC were bonded together with PVC glue and then sealed with a clear silicone adhesive. Leakage was minimized at the piping and sheet PVC contact points by inserting a 61 cm by 91.5 cm autoclavable 1.5 mm thick polypropylene bag (Cole-Parmer Instrument Company, Vernon Hills, IL, USA) into the tank. Extra polypropylene material was draped over the edge of the tank and affixed to the outer tank wall with white duct tape. Polypropylene liners, which were replaced when solution was replaced, were inserted into tanks to maintain a clean growth chamber.

Circular fitted lids were formed from sheet PVC to prevent unwanted evaporative water losses from inside the tank. Five holes of various diameters were bored into the lid of each tank. Three 2.5 cm holes were bored at equally spaced locations in the lid and were used for the placement of plant cuttings (see below). One 0.65 cm hole was used for the insertion of flexible aeration tubing, 0.635 cm in diameter. The final hole, 1 cm in diameter, was used as a pH and temperature monitoring port. When not being used, the monitoring port was sealed with a rubber stopper. Tanks and lids were insulated on the outside with a self-adhesive heat reflective barrier. The barrier consisted of a double

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wrapping of commercially available reflective insulation material. The reflective insulation was coated with a self-adhesive white contact paper to obtain an insulation value of R19. Tanks and lids were numerically labeled for tracking purposes.

Tanks were aerated by two 28.4 L minute^{-1} General Hydroponics dual diaphragm four valve high output air pumps (General Hydroponics, Sebastopol, CA, USA). Flexible clear aeration tubing was attached from the air pump to the lid of each tank by a series of aquarium gang valves. Several centimeters of clear tubing was pressed through a hole in each lid and was inserted over a portion of opaque rigid tubing (22 cm in length) extending towards the bottom of the tank. At the end of the rigid tubing a single aquarium air stone (2.5 cm in length) was attached. Rigid tubing was used to maximize the depth at which air was being supplied in the tank. Valves were deliberately turned so that air was released gently into the tank.

Plant Collection and Propagation

Two species of plants were collected for use in this study; they were *Salix exigua* Nutt. (native) and *Tamarix ramosissima* Ledebour (exotic). These plants were chosen for comparison because of their similarities in physiognomy and functional ecophysiology and because *Salix* is commonly replanted at locations where *Tamarix* has been removed. *Salix* and *Tamarix* pole cuttings were collected on April 10, 2004 (Julian Day 101) from the Meadow Valley Wash (708417 easting, 4080463 northing, 2000 m, Universal Transverse Mercator, North American Datum 1983) in Lincoln County, Nevada. Salvaging activities were allowed by vegetative material free use permit number NV-040-VG4-211 provided by the U.S. Bureau of Land Management, Caliente, Nevada field

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station. More than 100 pole cuttings each with diameters less than 1.5 cm were collected from the upper stems of several live plants growing along the border of the wash. Stems were cut to approximately 0.4 m long sections with a garden shearing tool and were stripped of lateral branches and leaves by hand. The bottom end (i.e., the root end) of the pole was cut at a 45° angle and the top end was cut perpendicular to the stem's shaft so that they would be planted in the hydroponic tanks in the proper orientation. Poles were fully submerged in reverse osmosis water until it was time to plant them.

Pole cuttings were planted on April 12, 2004 (Julian Day 103) into hydroponic tanks. Each of the 36 tanks was fitted with three pole cuttings of the same species. Approximately one-third of each pole cutting was placed below the lid of the tank. Stems were supported by clasping large rubber stoppers around the middle of the cutting and placing them into the 2.5 cm openings in the lid. The large rubber stoppers were prepared by drilling 1 cm holes through the center. A cut was then made parallel to the vertical axis so that the stopper could be easily clasped around a pole cutting. Cotton balls (100% cotton) were wrapped around the pole cutting prior to being fitted with a rubber stopper. This was done in order to minimize vertical and horizontal movement of the plant as well as to minimize evaporative water loss from within the tank. The planted holes were labeled either A, B, or C on the surface of the lid.

Prior to exposing *Salix* and *Tamarix* cuttings to perchlorate, pole cuttings were nourished with a modified one-half strength Hoagland's solution (Table 1). Hoagland's solution was prepared weekly by combining known concentrations of nutrient standards with reverse osmosis water in 120 L capacity reservoirs. Nzengung and Wang (2000) noted that a significant difference in perchlorate degradation was evident in experimental

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Table 1. Modified one-half strength Hoagland nutrient solution used to nourish*Salix exigua* and *Tamarix ramosissima* stems.

			Modified ½ Strength Hoagland Solution		
Compound Name	Molecular Formula	Molecular Weight	Grams Diluted to Make 1 Liter Stock Solution	Milliliters Used per Liter of Nutrient Solution	Milligrams of Compound per Liter of Nutrient Solution
Calcium sulfate	$CaSO_4$	136.14	136.14	2.5	340.35
Potassium nitrate	KNO_3	101.1	101.1	0.8	80.88
Ammonium dihydrogenphosphate	$NH_4H_2PO_4$	115.03	115.03	0.5	57.515
Potassium sulfate	K_2SO_4	174.26	174.26	0.85	148.121
Magnesium sulfate heptahydrate	$MgSO_4 \cdot 7H_2O$	246.47	246.47	1	246.47
Ethylenediaminetetraacetic acid iron(III) sodium salt	$C_{10}H_{12}N_2NaFeO_8$	367.05	10	0.5	5
Boric acid	H_3BO_3	61.83	2.86	0.5	1.43
Zinc sulfate heptahydrate	$ZnSO_4 \cdot 7H_2O$	287.56	0.22	0.5	0.11
Copper(II) sulfate pentahydrate	$CuSO_4 \cdot 5H_2O$	249.69	0.8	0.5	0.4
Manganese(II) chloride tetrahydrate	$MnCl_2 \cdot 4H_2O$	125.84	0.81	0.5	0.405
Molybdic acid	H_2MoO_4	161.95	0.028	0.5	0.014

tanks containing *Salix* spp. that were exposed to different sources of nitrogen and that increased nitrates inhibited perchlorate degradation. *Salix* spp. exposed to nitrate (NO_3^-) nutrient solutions were less efficient than *Salix* spp. exposed to urea ($[NH_2]_2CO$) or ammonium (NH_4^-) nutrient solution. Nzengung and Wang (2000) hypothesized that nitrate ions competed with perchlorate ions as root level terminal electron acceptors. For these reasons, and considering the potential for using *Tamarix* and *Salix* to remediate perchlorate near the Las Vegas Wash, nitrate and sulfate concentrations in the nutrient solution were modified (nitrate 465.1 mg L^{-1} and sulfate 192.1 mg L^{-1} before modification; nitrate 49.6 mg L^{-1} and sulfate 672.5 mg L^{-1} after modification) to be closer to the concentrations found in the Las Vegas Wash (nitrate 14.3 mg L^{-1} and sulfate 631 mg L^{-1}).

Treatment Group Design and Setup

Experimental treatment began on June 5, 2004 (Julian Day 154) with 26 hydroponic tanks. Tanks were divided by treatment and species, with 12 tanks containing *Salix* cuttings, 12 tanks containing *Tamarix* cuttings, and two tanks containing no cuttings. During the pre-treatment establishment period, some cuttings died and therefore tanks used in this study did not each have three cuttings. An attempt was made to have the same number of cuttings represented in each treatment group. However, since root systems had become intertwined, which made plant separation impossible, some tanks had more cuttings than others. Four treatment groups were used to determine the removal of perchlorate mass from hydroponic solution and the fate of that perchlorate in the stems, roots, and leaves of *Tamarix* and *Salix*. This included three groups exposed to

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perchlorate and one not exposed (i.e., a control group). A fifth, untreated, group was used as a blank control group, which consisted of two tanks and no planted cuttings. These tanks were used to evaluate evaporative losses. The 2.5 cm holes in the lids of the blank group tanks were simply plugged with solid rubber stoppers.

A total of 26 tanks were used for experimental treatment purposes. Treatment groups were exposed to three different concentrations of ammonium perchlorate for each of the plant species. Molar standards of ammonium perchlorate (Sigma-Aldrich, St. Louis, MO, USA) were prepared weekly to be used for each treatment. Perchlorate concentrations used in the treatment groups included an environmentally relevant concentration as well as concentrations that have been found in previous studies to phytoaccumulate (Nzengung et al. 1999). The first treatment group, exposed to 0.02 mg L^{-1} of ammonium perchlorate, consisted of three *Tamarix* tanks (4 cuttings total) and three *Salix* tanks (8 cuttings total). The second treatment group, exposed to 10 mg L^{-1} of ammonium perchlorate, consisted of three *Tamarix* tanks (4 cuttings total) and three *Salix* tanks (8 cuttings total). The third treatment group, exposed to 100 mg L^{-1} of ammonium perchlorate, consisted of three *Tamarix* tanks (4 cuttings total) and three *Salix* tanks (7 cuttings total). The control group (0 mg L^{-1}), consisting of three *Tamarix* tanks (4 cuttings total) and three *Salix* tanks (8 cuttings total), was not exposed to ammonium perchlorate. In addition, each of the blank tanks was not exposed to ammonium perchlorate.

Tanks were exposed for a period of four consecutive five-day periods (a total of 20 days). Hydroponic nutrient solution, as described above, was prepared for treated and untreated tanks on the first day of each period. Solutions were prepared for each

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treatment group by mixing known concentrations of nutrient and ammonium perchlorate standards with reverse osmosis water in separate 120 L capacity reservoirs. Separate reservoirs were used to maintain concentrations at expected levels. Control and blank tanks were nourished with perchlorate free nutrient solution provided from a common reservoir. Nutrient solutions were transferred from reservoirs to the hydroponic tanks with several electrically powered 5 cm submersible pumps attached to 1 cm clear flexible vinyl tubing. Hydroponic tanks were filled with nutrient solution to within 2.5 cm of the tanks top edge. Each ammonium perchlorate exposure group had its own submersible pump that was used exclusively for that treatment. Pumps and tubing were regularly rinsed with reverse osmosis water. At the end of each five-day period tanks were drained and then refilled with fresh solution. Perchlorate solution was drained into several 208 L metal drums and was disposed of by the University of Nevada, Las Vegas Risk Management and Safety Department.

Treatment Solution Monitoring, Sampling, and Analyses

On the first and final day of a sampling period, tank and solution mass (kg) was determined gravimetrically with an Ohaus CD-11 scale (Ohaus Corporation, Pine Brook, NJ, USA). Cuttings, which were attached to the lid by the rubber stoppers, were removed from the tank and placed on a clean polypropylene lined support structure prior to mass measurements. Solution volume (mL), recorded as mass, was determined by subtracting empty tank mass from full tank mass. Mass measurements were recorded to calculate solution removal for each period. Tanks were checked periodically to maintain static

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water levels. If water levels in a tank dropped below 2.5 cm from the top edge, perchlorate-free nutrient solution (control solution) was added from a clean 1 L capacity graduated cylinder. Volume, to the nearest mL, was recorded for tanks that required solution. Final mass balance calculations incorporated daily solution additions. On the final day of each period just prior to sampling, water was not added to the tanks.

Tanks were monitored daily for pH and temperature (°C) with a digital pH/Temp/mV/ISE meter (Beckman Coulter, Inc. Fullerton, CA, USA). The pH of the solution was maintained near 7.0 by either adding 1M H_2SO_4 or 10% KOH solution.

Water samples were collected from all tanks at the beginning and end of each period. Clean 100 mL sample containers were submerged into tank solution when the tanks were first filled then again before solution was discarded. Solution sample volumes were calculated by subtracting full tank mass after sampling from full tank mass before sampling. Final mass balance calculations incorporated sample volume removal. Water samples were collected daily from solution preparation reservoirs. All samples were labeled and refrigerated until analyzed.

All water samples were analyzed for the presence of perchlorate, nitrate (NO_3^-), chloride (Cl^-), and sulfate (SO_4^{2-}). Perchlorate samples were analyzed with an Ion Chromatograph (IC) by EPA method 314.0 developed by Hautman et al. (1999). EPA method 314.0 describes the use of an ion chromatograph for determining the concentration of perchlorate in water. Hautman et al. (1999) state that "the analytical system complete with eluent reservoirs, an ion chromatographic pump, injection valves, both guard and analytical separator columns, suppressor, conductivity detector, and a computer based data acquisition system" must be used. Nitrate, chloride, and sulfate

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samples were analyzed with a DX-120 IC (Dionex Corporation, Sunnydale, CA, USA). Mass balance calculations were performed for some constituents. In order to calculate a mass balance for samples that were analytically below the detection limit, one-half the method detection limit (MDL) was used.

Plant Tissue Monitoring, Sampling, and Analysis

After *Tamarix* and *Salix* pole cuttings were planted on April 12, 2004 (Julian Day 103), they were checked daily for newly developing roots, buds, and leaves. Dates were recorded when new vegetative material was first observed. Once experimental treatments were initiated, plants were visually monitored daily for stress, as indicated by leaf turgidity, curling, and discoloration.

On June 25, 2004 (Julian Day 177), after 20 days of exposure, plants were salvaged from their tanks so that tissues could be analyzed for the presence of perchlorate. Plants were removed from tank lids by clipping the main stem just above the rubber stopper. This effectively sectioned the plant into either above lid biomass or below lid biomass. Plants were allowed to air dry so that no visible water was present on the once submerged stems and roots. Wet weights for the various plant parts (Figures 2-3) were then recorded to the nearest tenth of a milligram on a XD-4KD scale (Denver Instruments Company, Arvada, CO, USA).

The main stem portion below a tank's lid was cut from the small roots that had grown since the stems were originally planted. Cumulative small root mass was recorded since root systems from one cutting could not be untangled from those of another cutting

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Figure 2. Photograph of *Salix exigua* plant parts before and after grinding.

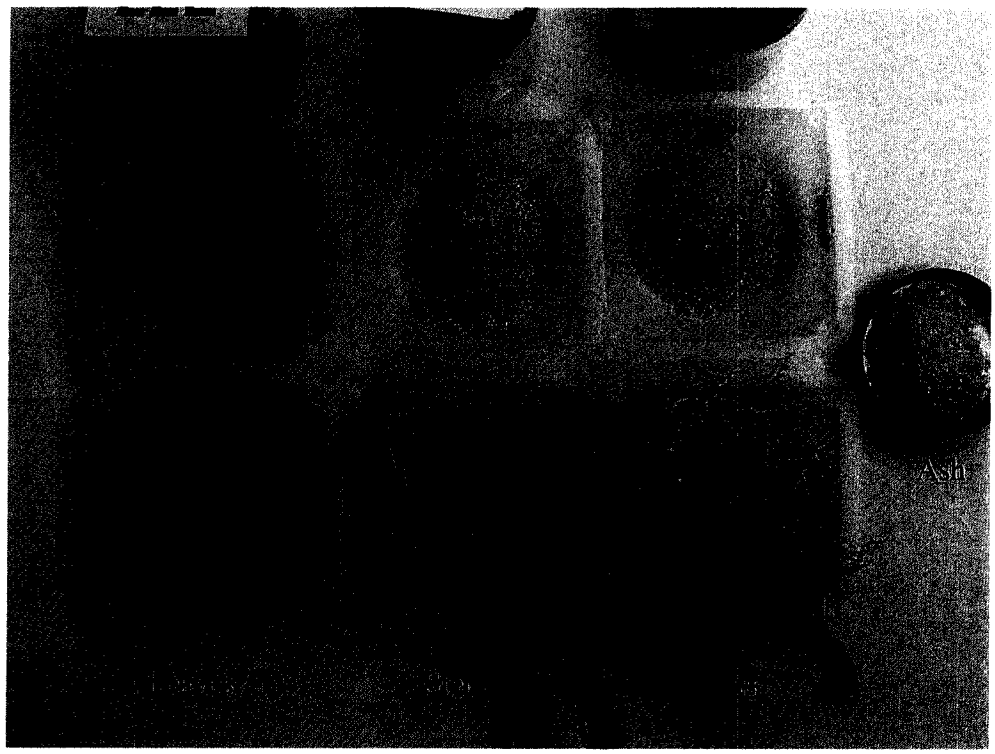

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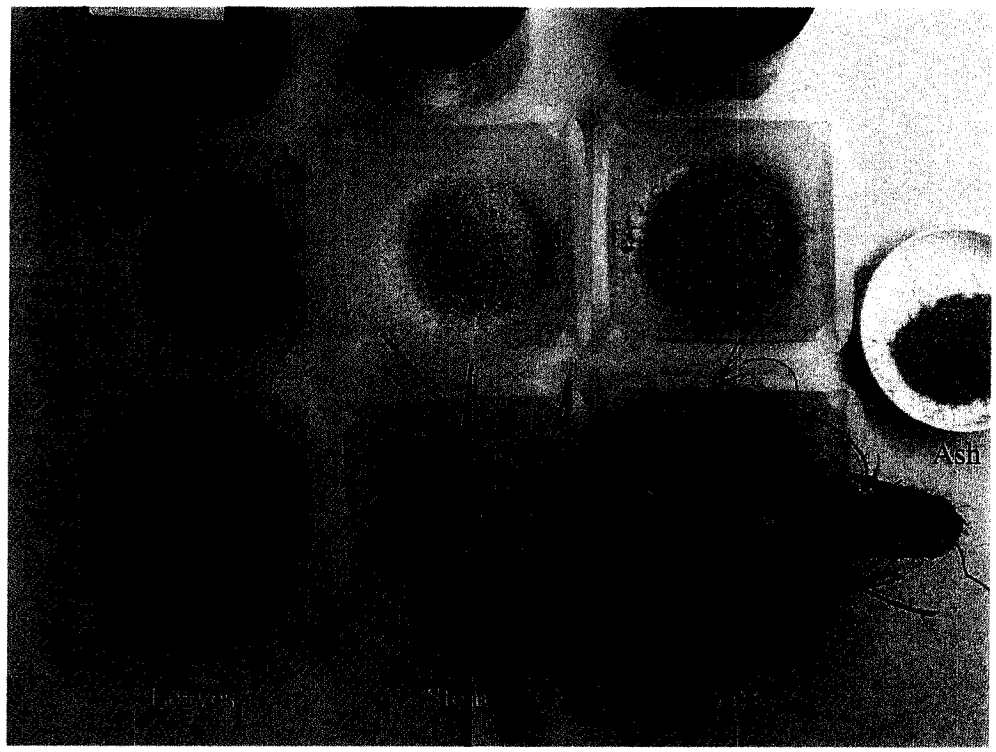
Figure 3. Photograph of *Tamarix ramosissima* plant parts before and after grinding.



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in the same tank. Mass was recorded for the main stem of the plant that was below the lid of the tank, termed "big roots". Biomass above the tank's lid was weighed then sectioned into a main stem portion (i.e., big stems) and a leaf-stem portion. Leaves were not separated from stems at this time. The main stem above the lid was cut from the new growth and weighed, and the remaining leaf-stem mass was calculated. When more than a single plant was in a tank, plant tissue samples were combined.

To evaluate the potential secretion of perchlorate salts onto *Tamarix* and *Salix* leaves, the leaf-stem portions of plants were bathed in a known volume of reverse osmosis water. Because of the high solubility of perchlorate, salts that had exuded onto the surface of leaves would easily be dissolved in solution. The solution bath was then transferred to labeled, clean 1 L sample containers. After the bath, leaf-stem material was left to air dry and then placed in paper bags. The solution bath was refrigerated until it was analyzed for perchlorate by EPA method 314.0.

Tissue samples were oven dried in an oven at 60°C for 48 hours. Oven-dry mass was recorded for small root, big root, and big stem sections. After oven drying, leaf-stem fractions were separated into leaf and stem samples. Because *Tamarix* leaves are small, bract like, and difficult to separate from stems, leaf samples included stem material that was less than 1 mm in diameter. *Salix* leaves and stems were much easier to separate; however in order to remain consistent, *Salix* leaf material (i.e., leaf and petiole) also included stems less than 1 mm in diameter. Oven-dry weight was recorded for the separated stem and leaf material. After plant tissues were weighed, they were stored in paper bags at approximately 24°C until analyzed.

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Tissue samples were divided into three categories – leaves, stems, and roots – and were analyzed for the presence of perchlorate (see below for analytical method). Leaf samples consisted of leaves, petioles, and stems less than 1 mm in diameter. Stem samples partly consisted of new stem growth in addition to the main stem above the lid (i.e., a portion of the original cutting that was planted, big stems). Root samples, like that of the stem samples, consisted of a portion of the main stem that was below the lid (i.e., big roots) in addition to the newly grown roots. Because of the relatively heavy mass of the main stem, and its potential importance for closing a perchlorate mass balance, it was included in the samples. Root and stem samples, however, were combined on a proportionally based mass ratio. For example, if new stem growth weighed 1 g and the main stem above the lid weighed 9 g, then the analyzed stem sample would consist of 10% new stem growth mass and 90% main stem above lid mass. These samples were separated in this way because laboratory costs precluded the direct analysis of all tissue samples yet it was important to be able to calculate perchlorate tissue mass for the entire plant in addition to each plant part.

In order to partially meet the study's objectives for determining if perchlorate remains in burned leaf material, fractions of leaf samples were burned. Fractions of dried leaf sample (5-15 g) were weighed and placed in ceramic boats. Samples were then placed in a Thermolyne furnatrol I furnace oven (Thermolyne Corporation, Dubuque, IA, USA) and baked at 600°C for 6 hours until only ash remained. Typically, leaf material combusted within the first few seconds of being placed in the oven. The resulting ash was then reweighed and placed in labeled, clean 40 mL glass jars.

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Leaf, stem, and root samples were frozen overnight at -70°C in a BioFreezer (Forma Scientific, Marietta, OH, USA). Samples were then placed for a minimum of three hours in a Freezone 18 Liter Console Freeze Dry System (Labconco Corporation, Kansas City, MO). Samples were weighed before and after freeze-drying for percent moisture calculations. Samples were then ground to a powder with a Wiley Mini-Mill and passed through a 0.85 mm sieve (Thomas Scientific, Swedesboro, NJ, USA). Ash samples did not require freeze-drying or milling since the furnace oven burning process was sufficient to break down tissues. Small stem and big stem milled material were mixed together according to a mass proportional calculation (see discussion above). Small roots and big roots were similarly mixed. Leaf, stem, and root milled samples and ash samples were mixed in 33 mL cells with 100% deionized (DI) 18.1 MΩ water and ottowa sand and extracted with an Accelerated Solvent Extraction System (ASE 200, Dionex Corporation, Sunnyvalle, CA, USA) at 100°C and 10,342 Kilopascals for a static time of 20 minutes.

Prior to perchlorate analysis, leaf, stem, root, and ash sample solutions were prepared by sequential elution through an OnGuard II Ba and an OnGuard II H cartridge (Dionex Corporation, Sunnyvale, CA, USA). Cartridges were conditioned prior to use by flushing and discarding 10 mL of DI water. Samples were eluted at a flow rate of 2 mL $minute^{-1}$ and the first 6 mL of eluent was discarded. Depending on the analytical method employed, 2-10 mL of eluent was saved for analysis. IC with Suppressed Conductivity Detection was used for the determination of perchlorate and method parameters were based on EPA method 314.0. A Dionex DX 500 IC system equipped with a GP50 gradient pump was connected to a 50 mm by 4 mm (IonPac® AG16-HC) guard column and a 250 mm by 4 mm (IonPac® AS16-HC) analytical column. A 20-minute isocratic

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elution with 50 mmol sodium hydroxide was used for separation of the perchlorate ion and continuous suppression at 300 mA was carried out using a Dionex ASRS-Ultra II 4 mm suppression system. A Dionex AS40 autosampler with 5 mL cartridges was used for the injection of 1 mL samples into the IC column. For detection, a Dionex CD20 conductivity detector was employed. Perchlorate concentrations in the samples were determined against an external calibration curve with a 5-100 $\mu g\ L^{-1}$ range. In order to calculate a mass balance, one-half the method detection limit (MDL) was used for samples that were analytically non-detect.

Statistical Methods

All statistics were done with SigmaStat for Windows Version 3.11 (Systat Software, Inc., Point Richmond, CA, USA) or Microsoft Office Excel 2003 (Microsoft Corporation, Redmond, WA, USA). Standard descriptive statistics were initially used to evaluate the data. Descriptive statistics used included the minimum, maximum, mean, and standard error. Hypothesis testing was conducted with parametric tests. Since parametric tests assume that samples come from normally distributed populations with the same variance, the Kolmogorov-Smirnov normality test and Levene Median test were used to satisfy these assumptions. Square root and natural log transformations were used to linearize samples that did not meet parametric test assumptions. Samples for all parametric tests met normality and equal variance assumptions unless noted. One Way and Two Way Analysis of Variance (ANOVA) were used to compare the effect of a single factor or two different factors on the mean of two or more groups. Post hoc multiple comparison

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testing was performed with the Holm-Sidak test. Tests were conducted with $\alpha = 0.05$ and were considered significant if the $P < 0.05$.

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CHAPTER 4

RESULTS AND DISCUSSION

Pre-Treatment Growth

Within seven days (Julian Day 110) of planting stems into hydroponic tanks, most cuttings exhibited leaf and root development. Typically, leaves and roots developed quicker on *Salix* cuttings, with 90% of stems having leaves by the seventh day. Additionally, roots in excess of 7 cm were observed on *Salix* cuttings on Julian Day 110. On the same day, *Tamarix* cuttings mostly exhibited only bud and root nodule swelling. By Julian Day 112, however, most *Tamarix* cuttings had developed leaves and roots, with maximum root lengths exceeding 11 cm, with some lateral root development. On the same day, *Salix* cuttings had roots greater than 13 cm and twice pinnate root laterals were forming. By Julian Day 113, 100% of *Salix* cuttings had leaves while only 65% of them had roots. In contrast, 80% of *Tamarix* cuttings had leaves and only 43% had roots on the same day. Ten *Tamarix* stems (19%) were observed dead by Julian Day 117 and interestingly, salt crystals were observed on the leaf surface for *Tamarix* tank 14 plant A. On the same day 100% of *Salix* stems were alive and exhibited robust leaf and root growth with some secondary stems forming as well.

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Pre-Treatment Evapotranspiration

Cuttings were grown in hydroponic solution for a period of 54 days prior to exposure. During this period, weekly evapotranspiration (ET) increased dramatically. *Salix* ET increased from 40.7 mL day^{-1} for the week ending on Julian Day 108, to more than 950 mL day^{-1} for the week ending on Julian Day 143 (Figure 4). On Julian Day 143, 150, and 157, *Salix* plant mass (i.e., roots and stems) was culled back to reduce evapotranspirative loss and provide space in the tank because several *Salix* tanks were completely full of roots. *Tamarix* ET increased from 34.3 mL day^{-1} for the week ending on Julian Day 108 to more than 710 mL day^{-1} on the week ending on Julian Day 157. *Tamarix* biomass did not need to be culled back.

Greenhouse Conditions

The experiment began on Julian Day 157 and ended on Julian Day 177. Greenhouse temperatures, relative humidity, and carbon dioxide temporally varied (Figure 5). Typically the highest air temperatures were recorded between 1200 and 1500 hours, with a mean of 33.0°C at 1300. Relative humidity (RH) was lowest during the period from 1500 to 1700 hours, with the lowest mean RH recorded at 1500 hours of 14.9%. Carbon dioxide was lowest between 1200 and 1500 hours, with values as low as 377 μmol mol^{-1}. Carbon dioxide was greatest just before sunrise with a mean value of 406 μmol mol^{-1} at 0500 hours.

Mean weekly tank temperatures did not vary significantly ($P > 0.05$) between treatments or species (Table 2). The mean temperature for all periods, treatments, and species combined was 26.36 °C. Generally, control tanks had the lowest mean weekly

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Figure 4. Mean weekly evapotranspiration for*Salix exigua* and *Tamarix ramosissima* plants for the 54 day period before plants were exposed to ammonium perchlorate. * Root and stem material was culled back.

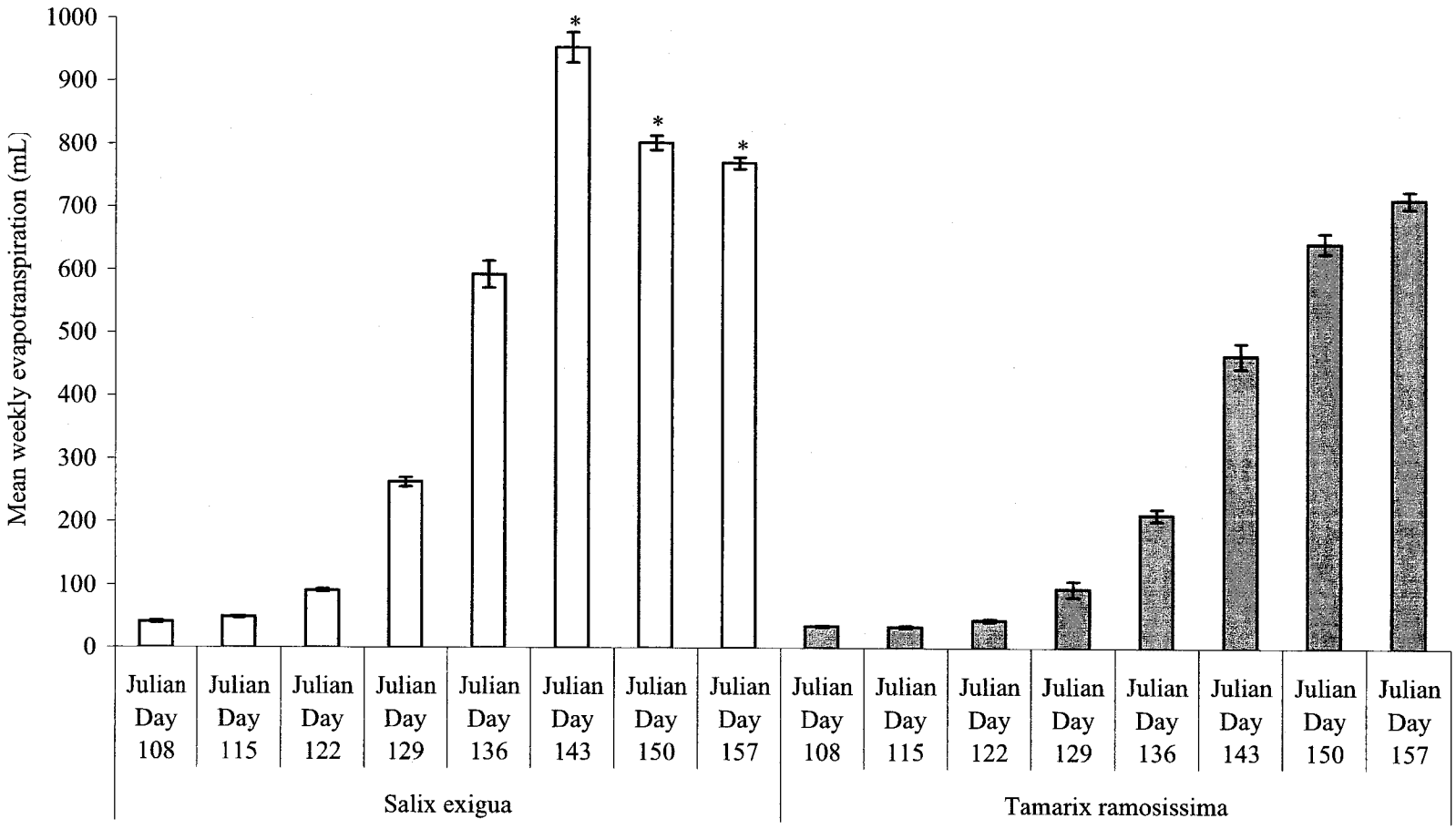

Figure 5. Hourly mean a) air temperature (°C), b) relative humidity (%), and c) carbon dioxide (μmol mol^{-1}) ± SE in the greenhouse during June 5-25, 2004.

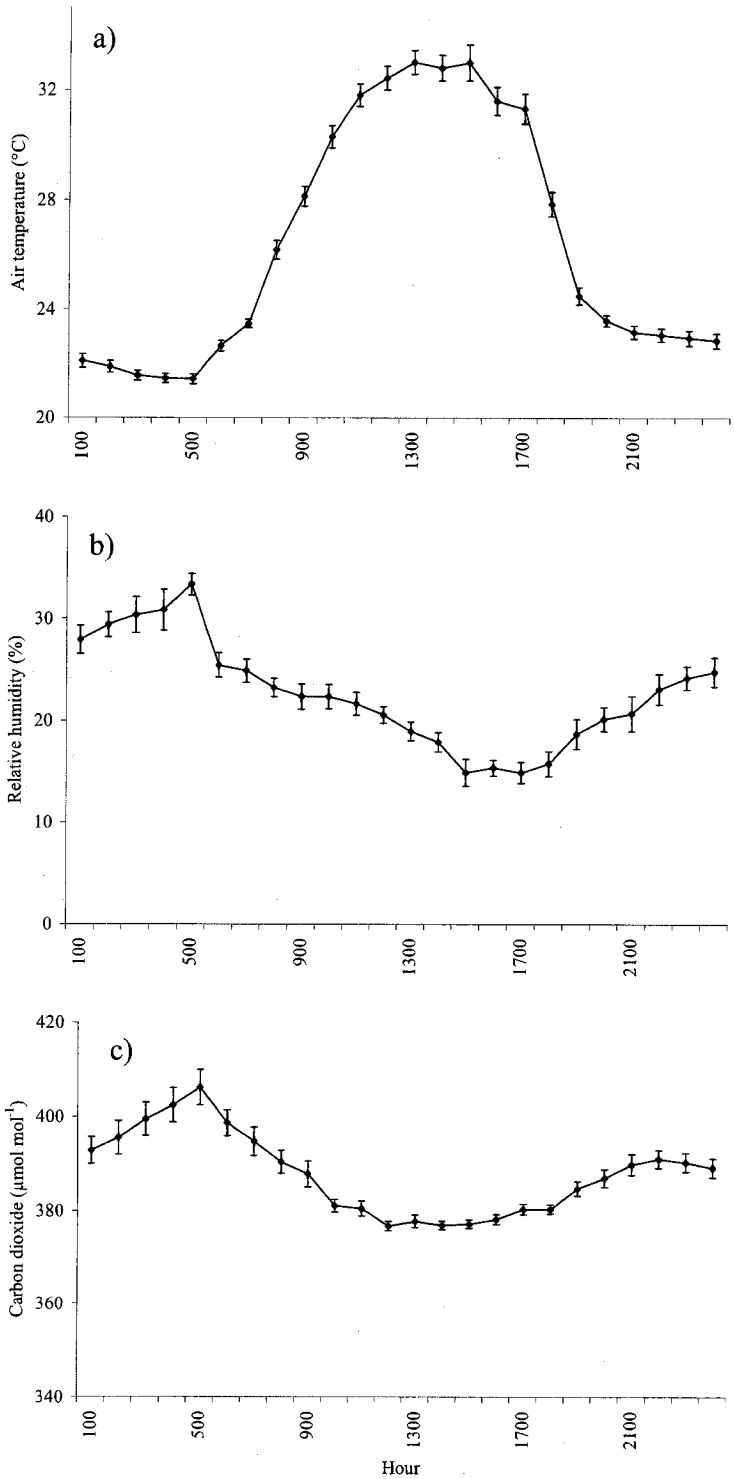

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Table 2. Mean nutrient solution temperature (°C) for *Salix exigua* and *Tamarix ramosissima* cuttings planted in hydroponic solution for four consecutive 5-day periods. Significant differences ($P < 0.05$) were not detected between species or treatments.

		Treatment			
Period	Species	0 mg L^{-1}	0.02 mg L^{-1}	10 mg L^{-1}	100 mg L^{-1}
1	*Salix exigua*	24.8	26.5	27.2	26.5
	Tamarix ramosissima	25.6	26.5	26.8	26.9
2	*Salix exigua*	24.8	26.1	26.5	26.3
	Tamarix ramosissima	25.1	26.0	25.9	26.4
3	*Salix exigua*	25.8	26.4	27.3	26.6
	Tamarix ramosissima	26.0	26.5	27.0	27.1
4	*Salix exigua*	25.6	26.7	27.3	26.8
	Tamarix ramosissima	25.9	26.7	26.9	27.1

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temperatures while the 10 mg L^{-1} group had the highest. The difference, however, was generally less than two degrees. Thermal insulation material used to protect the tanks was sufficient to maintain water temperatures on average 7.1°C below air temperatures in the greenhouse.

Tank pH was generally near neutral with a typical range of 6.5-7.5. The mean pH for all periods, treatments, and species combined was 7.1. There were no significant differences ($P > 0.05$) between weekly pH for species within the different treatments. There was, however, a minor treatment effect. During period 1, the 100 mg L^{-1} *Salix* group was significantly lower ($P < 0.05$) than the other *Salix* treatments (Table 3).

Evaporation and Evapotranspiration

Water loss due to evaporation, transpiration, or both was recorded daily and is described as evapotranspirative loss (ET) or evaporative loss. Water lost from unplanted tanks (i.e., blank tanks) was recorded to determine the amount of water loss that was likely due to evaporation. Blank tanks showed a decline in mean weekly evaporation loss over the experiment. Mean tank evaporation loss was greatest during the first period (123 mL day^{-1}) but declined to 22 mL day^{-1} by the last period. A total of 2.72 L were evaporated out of both blank tanks for the 20-day period which shows that hydroponic tanks were not completely sealed. Water loss from the tanks could have occurred at the lid seal or around the rubber stoppers in the lid. Since water was lost through evaporation, perchlorate concentration data at the end of each five day exposure period would likely be higher than expected due to evapoconcentration effects. Moreover, tanks were not replenished with nutrient solution on the last day of each period prior to water

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Table 3. Mean tank solution pH for *Salix exigua* and *Tamarix ramosissima* cuttings planted in hydroponic solution for four consecutive 5-day periods. Different superscript letters indicate significant differences ($P < 0.05$) between treatments.

		Treatment			
Period	Species	0 mg L^{-1}	0.02 mg L^{-1}	10 mg L^{-1}	100 mg L^{-1}
1	*Salix exigua*	7.3^{a}	7.0^{a}	7.0^{a}	6.4^{b}
	Tamarix ramosissima	7.1^{a}	7.0^{a}	7.1^{a}	6.6^{a}
2†	*Salix exigua*	7.3^{a}	7.2^{a}	7.1^{ab}	6.9^{b}
	Tamarix ramosissima	7.3^{a}	7.3^{a}	7.2^{a}	7.0^{a}
3	*Salix exigua*	7.2^{a}	7.0^{a}	7.0^{a}	7.1^{a}
	Tamarix ramosissima	7.1^{a}	7.1^{a}	7.1^{a}	7.0^{a}
4	*Salix exigua*	7.1^{a}	7.1^{a}	7.1^{a}	6.9^{a}
	Tamarix ramosissima	7.2^{a}	7.2^{a}	7.1^{a}	7.0^{a}

† Data did not pass normality test.

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sampling, which would also result in higher than normal perchlorate concentrations in the final solution. Comparisons between initial and final solution concentrations, therefore, are not good indicators of perchlorate changes in the tanks.

Evapotranspiration increased throughout the experiment and varied between species and treatments (Figure 6). A total of 632.6 L of solution was lost from planted tanks, with 60% of the loss attributed to *Salix* tanks and 40% of the loss attributed to *Tamarix* tanks. *Salix* tanks lost 56.09 L over the first 5-day period, which increased to 131.49 L by the last 5-day period. *Tamarix* ET ranged from 47.08 L to 78.01 L over the same time. The 0.02 mg L^{-1} treatment had the lowest cumulative ET of 149.1 L and the 10 mg L^{-1} treatment had the highest, 168.1 L. Average ET for *Salix* tanks (31.78 L $tank^{-1}$) was significantly higher ($P < 0.05$) then *Tamarix* tanks (20.94 L $tank^{-1}$). The observed high water use by *Salix* and *Tamarix* was consistent with data reported by Sala et al. (1996), who showed that these species are luxurious water users when water is readily available, such as under high water table conditions. High water tables are represented here as an unlimited supply of solution delivered hydroponically.

Daily ET estimates calculated from mean five day ET measurements showed that the lowest ET occurred during the first 5-day period. During this period, the lowest ET for *Salix* tanks were in the 10 mg L^{-1} treatment group (882 mL day^{-1}) while the least evapotranspiring *Tamarix* tanks were in the control group (662 mL day^{-1}). There was a significant species interaction ($P < 0.05$), with the 5-day mean ET for *Salix* being greater than *Tamarix* for all period and treatment comparisons except during the first period (Table 4). No significant treatment interaction was observed because 5-day mean ET did

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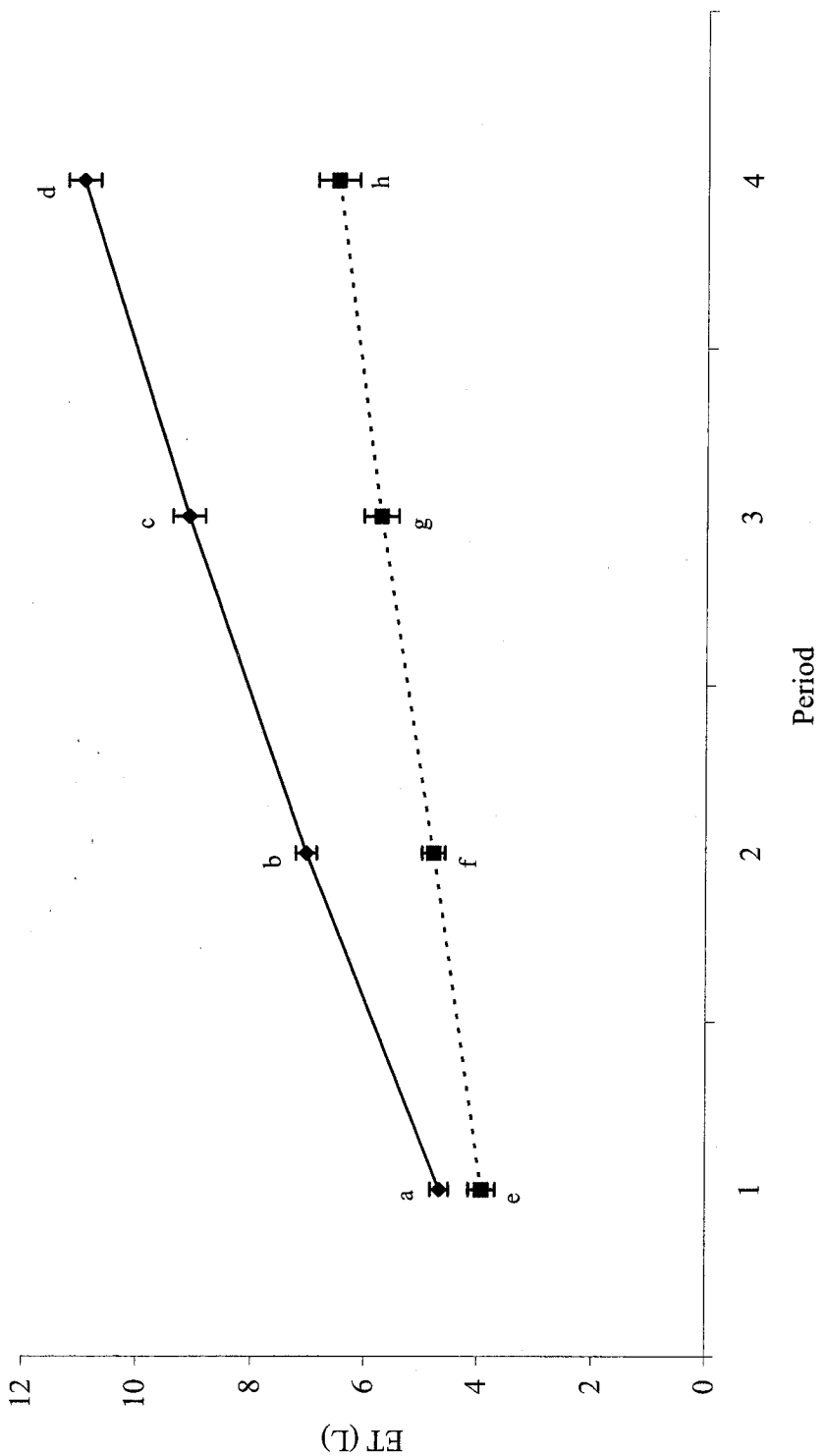

Figure 6. Mean evapotranspiration (L) ± SE for *Salix exigua* (solid line) and *Tamarix ramosissima* (dashed line) tanks for each 5-day period. Different superscript letters indicate significant differences ($P < 0.05$) between species and across treatments.

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Table 4. Mean evapotranspiration (L) from tanks planted with Salix exigua and Tamarix ramosissima cuttings for four consecutive 5-day periods. Different superscript letters indicate significant differences ($P < 0.05$) between species or treatments.

		Species	
Period	Treatment	*Salix exigua*	*Tamarix ramosissima*
1	0 mg L^{-1}	5.22^{a}	3.31^{b}
	0.02 mg L^{-1}	4.46^{a}	3.72^{a}
	10 mg L^{-1}	4.41^{a}	4.22^{a}
	100 mg L^{-1}	4.61^{a}	4.44^{a}
2	0 mg L^{-1}	7.09^{a}	4.36^{b}
	0.02 mg L^{-1}	6.65^{a}	4.61^{b}
	10 mg L^{-1}	7.29^{a}	5.20^{b}
	100 mg L^{-1}	7.13^{a}	4.98^{b}
3†	0 mg L^{-1}	8.76^{a}	4.94^{b}
	0.02 mg L^{-1}	8.29^{a}	5.63^{b}
	10 mg L^{-1}	9.47^{a}	6.47^{b}
	100 mg L^{-1}	9.90^{a}	5.89^{b}
4‡	0 mg L^{-1}	10.94^{a}	5.54^{b}
	0.02 mg L^{-1}	10.02^{a}	6.32^{b}
	10 mg L^{-1}	11.70^{a}	7.26^{b}
	100 mg L^{-1}	11.16^{a}	6.89^{b}

† Data did not pass equal variance test.

‡ Data did not pass normality test.

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not significantly differ ($P > 0.05$) between treatments when compared within periods and species.

Biomass

Oven-dry biomass (g) was determined at the end of the experiment for the pole cuttings that were originally planted in tanks as well as the new growth that had occurred after planting. Pole cutting biomass did not differ significantly ($P > 0.05$) between species, treatment, or treatment by species interactions (Table 5). Pole cutting size, therefore, was not a source of variability. Total plant biomass differed significantly ($P < 0.05$) between species but there was no difference between treatment groups. *Salix* biomass was significantly greater ($P < 0.05$) than *Tamarix* biomass within each treatment group comparison except the 100 mg L^{-1} treatment (Table 5). Also, mean biomass for *Salix* (115.5-133.3 g) exceeded *Tamarix* (79.1-89.1 g) for all treatment group comparisons. *Salix* plants were observed to have on average 45% more biomass than *Tamarix* plants. *Salix* control plants had the greatest mean biomass while *Tamarix* control plants had the lowest mean biomass, with their difference being approximately 68%. Mean daily biomass growth for *Salix* was 6.06 g day^{-1} while *Tamarix* was only 4.18 g day^{-1}. The lowest daily growth rate was for *Tamarix* control plants at 3.95 g day^{-1} and the greatest daily growth rate was for *Salix* control plants at 6.67 g day^{-1}. Since total biomass did not significantly differ ($P > 0.05$) between treatments for the same species, perchlorate exposure for 20 days did not appear to affect net photosynthetic performance, as measured by biomass accumulation of the two species.

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Table 5. Mean dry weight biomass (g) of *Salix exigua* and *Tamarix ramosissima* cuttings planted in hydroponic solution for a total of 20 days. Different superscript letters indicate significant differences ($P < 0.05$) between species or treatments.

		Species	
Matrix	Treatment	*Salix exigua*	*Tamarix ramosissima*
Leaf	0 mg L^{-1}	43.61^{a}	27.98^{bc}
	0.02 mg L^{-1}	37.60^{a}	31.56^{ac}
	10 mg L^{-1}	37.00^{a}	30.83^{ac}
	100 mg L^{-1}	41.07^{a}	33.83^{ac}
All stems	0 mg L^{-1}	41.42^{a}	29.94^{a}
	0.02 mg L^{-1}	39.12^{a}	28.33^{a}
	10 mg L^{-1}	37.93^{a}	32.70^{a}
	100 mg L^{-1}	38.50^{a}	32.97^{a}
Small stems	0 mg L^{-1}	18.59^{a}	11.09^{bc}
	0.02 mg L^{-1}	16.43^{a}	10.86^{bc}
	10 mg L^{-1}	19.47^{a}	15.13^{ac}
	100 mg L^{-1}	20.50^{a}	16.13^{ac}
All roots	0 mg L^{-1}	48.30^{a}	21.15^{b}
	0.02 mg L^{-1}	41.34^{a}	22.21^{b}
	10 mg L^{-1}	40.57^{a}	20.23^{b}
	100 mg L^{-1}	37.97^{a}	22.30^{b}
Small roots[†]	0 mg L^{-1}	22.59^{a}	7.74^{b}
	0.02 mg L^{-1}	20.09^{a}	8.32^{b}
	10 mg L^{-1}	19.70^{a}	7.87^{b}
	100 mg L^{-1}	22.80^{a}	8.03^{b}
Pole cutting (big stem and big root)	0 mg L^{-1}	48.53^{a}	32.26^{a}
	0.02 mg L^{-1}	43.94^{a}	31.36^{a}
	10 mg L^{-1}	39.33^{a}	29.93^{a}
	100 mg L^{-1}	33.17^{a}	31.10^{a}
All tissue	0 mg L^{-1}	133.33^{a}	79.07^{bc}
	0.02 mg L^{-1}	118.06^{a}	82.10^{bc}
	10 mg L^{-1}	115.50^{a}	83.77^{bc}
	100 mg L^{-1}	117.53^{a}	89.10^{ac}

[†] Data did not pass equal variance test.

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Total stem, total root, and leaf biomass was greatest for *Salix* with mean biomass values of 39.2 g, 42.0 g, and 39.8 g, respectively. *Tamarix* total stems, total roots, and leaves were between 5.2 and 27.1 g less than *Salix* plant material (Table 5). Total stem biomass was not significantly different ($P > 0.05$) between species within treatments or treatments within species. Mean values for *Tamarix* total root mass were significantly less ($P < 0.05$) than *Salix* total root mass. Most of the variation in total root mass was attributed to species comparisons while comparisons between treatments and treatments by species did not contribute significant variation (Table 5). Leaf biomass differed significantly ($P < 0.05$) between species in the control treatment but not in other treatments. Leaf biomass was not significantly different ($P > 0.05$) between treatments or treatments within species.

Stem and root biomass that had grown since pole cuttings were planted in the hydroponic tanks are described as small stem and small root fractions. Small stem biomass differed significantly ($P < 0.05$) between species only within the control and 0.02 mg L^{-1} treatments. Small root biomass differed significantly ($P < 0.05$) between species within all treatment group comparisons. Small roots weighed substantially less for *Tamarix* than for *Salix*, with their difference being greater than 11 g for each treatment group comparison. Since there was no treatment effect, it appears that these two species differ when it comes to resource allocation for roots and stems and the maintenance of root:shoot ratios.

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Perchlorate Removal – Solution Data

When tank solution was filled (S_i) at the beginning of each period, the mean perchlorate concentration deviated from the target concentration. For the 0.02 mg L^{-1} group, perchlorate concentrations ranged from 0.01 to 0.03 mg L^{-1} and did not differ significantly ($P > 0.05$) across species (Table 6). For the 10 mg L^{-1} group, S_i perchlorate concentrations ranged from 7.73 to 9.47 mg L^{-1} for *Salix* and *Tamarix*, with no significant difference ($P > 0.05$) between species for all periods (Table 6). The range in S_i for the 100 mg L^{-1} for all periods was from 76.00 to 103.00 mg L^{-1}. In the 100 mg L^{-1} group there was a significant difference ($P < 0.05$) with S_i between *Tamarix* and *Salix* during period two. There were no significant differences ($P > 0.05$) for species comparisons in the other three periods. Perchlorate was not detected in S_i for control or blank tanks at a MDL of 0.004 mg L^{-1}.

After each 5-day period, the concentration of perchlorate found in the final solution (S_f) was generally greater than the S_i. This was most likely due to evapoconcentration in the tanks because water was not added on the last day of the period prior to sampling. *Salix* mean S_f concentrations for the 0.02 mg L^{-1}, 10 mg L^{-1}, and 100 mg L^{-1} groups were 0.02 mg L^{-1}, 9.7 mg L^{-1}, and 100.8 mg L^{-1}, respectively. The *Tamarix* 0.02 mg L^{-1}, 10 mg L^{-1}, and 100 mg L^{-1} groups had S_f mean concentrations of 0.02 mg L^{-1}, 8.9 mg L^{-1}, and 97.5 mg L^{-1}, respectively. Perchlorate concentration only differed significantly ($P < 0.05$) between species during period three at the 0.02 mg L^{-1} and 100 mg L^{-1} treatments (Table 6). Significant differences that are reported here may be caused by differences in ET between the species.

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Table 6. Mean perchlorate concentration (mg L^{-1}) in initial solution (S_i) and final solution (S_f) from hydroponic tanks planted with *Salix exigua* and *Tamarix ramosissima* that were exposed to four ammonium perchlorate treatments over four consecutive 5-day periods. Different superscript letters indicate significant differences ($P < 0.05$) between species within each solution type. ND = not detected at method detection limit of 0.004 mg L^{-1}.

		S_i		S_f	
Period	Treatment	*Salix exigua*	*Tamarix ramosissima*	*Salix exigua*	*Tamarix ramosissima*
1	0 mg L^{-1}	ND	ND	ND	ND
	0.02 mg L^{-1}	0.03^a	0.02^a	0.03^a	0.03^a
	10 mg L^{-1}	8.50^a	8.13^a	9.60^a	8.10^a
	100 mg L^{-1}	84.0^a	93.0^a	93.33^a	94.67^a
2	0 mg L^{-1}	ND	ND	ND	ND
	0.02 mg L^{-1}	0.03^a	0.02^a	0.03^a	0.03^a
	10 mg L^{-1}	8.67^a	9.47^a	10.67^a	9.83^a
	100 mg L^{-1}	76.00^a	94.67^b	95.00^a	100.00^a
3	0 mg L^{-1}	ND	ND	ND	ND
	0.02 mg L^{-1}	0.02^a	0.02^a	0.02^a	0.01^b
	10 mg L^{-1}	9.07^a	9.10^a	11.00^a	8.97^a
	100 mg L^{-1}	103.00^a	99.00^a	136.67^a	106.67^b
4	0 mg L^{-1}	ND	ND	ND	ND
	0.02 mg L^{-1}	0.01^a	0.01^a	0.01^a	0.01^a
	10 mg L^{-1}	7.73^a	8.63^a	7.40^a	8.60^a
	100 mg L^{-1}	85.67^a	82.33^a	78.33^a	88.67^a

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Perchlorate mass comparisons provide a clear summary of perchlorate removal per tank. Perchlorate mean S_i mass differed between treatments, periods, and species although not always significant. Mean perchlorate S_i mass for the 0 mg L^{-1}, 0.02 mg L^{-1}, 10 mg L^{-1}, and 100 mg L^{-1} treatments were 0.015 mg, 0.15 mg, 64.4 mg, and 666.8 mg, respectively. Blank tanks had low mean mass values (S_i = 0.015 mg of perchlorate), similar to the 0 mg L^{-1} treatment. Significant differences ($P < 0.05$) were detected between species at the 10 mg L^{-1} treatment during period four and the 100 mg L^{-1} treatment during period two (Table 7). Other comparisons were not significantly different ($P > 0.05$). Differences in initial solution mass do not appear to be a result of tank volume differences; rather, they appear to be a result of initial solution concentration differences.

Mean S_f mass varied between treatments, species, and periods (Table 7). Interestingly, significant differences ($P < 0.05$) were detected between species at all periods for the 0 mg L^{-1} treatment. Other significant differences between species were also detected. In order to compare 0 mg L^{-1} treatments, one-half the MDL was used for mass calculations. Significant differences reported here for the 0 mg L^{-1} treatment is a result of not adding water to tanks on the last day of the period and does not represent true differences in removal.

A mass balance was calculated for each tank by adding together the total mass inputs of perchlorate, which was derived from solution concentration data, and subtracting out the remaining perchlorate mass in the tank also calculated from solution data. The remaining mass (i.e., the mass balance) consists of perchlorate mass that was taken up or degraded by the plants. Therefore, these data cannot identify how perchlorate was

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Table 7. Mean perchlorate mass (mg) in initial solution (S_i) and final solution (S_f) from hydroponic tanks planted with *Salix exigua* and *Tamarix ramosissima* that were exposed to four ammonium perchlorate treatments over four consecutive 5-day periods. Different superscript letters indicate significant differences ($P < 0.05$) between species within each solution type.

		S_i		S_f	
Period	Treatment	*Salix exigua*	*Tamarix ramosissima*	*Salix exigua*	*Tamarix ramosissima*
1	0 mg L^{-1}	0.016^a	0.015^a	0.013^a	0.014^b
	0.02 mg L^{-1}	0.23^a	0.14^a	0.21^a	0.20^a
	10 mg L^{-1}	62.53^a	60.93^a	59.29^a	53.01^a
	100 mg L^{-1}	622.36^a	684.28^a	575.77^a	603.78^a
2	0 mg L^{-1}	0.016^a	0.015^a	0.011^a	0.013^b
	0.02 mg L^{-1}	0.21^a	0.13^a	0.17^a	0.19^a
	10 mg L^{-1}	65.00^a	72.39^a	55.03^a	57.39^a
	100 mg L^{-1}	569.66^a	717.93^b	487.24^a	587.60^b
3	0 mg L^{-1}	0.015^a	0.015^a	0.009^a	0.013^b
	0.02 mg L^{-1}	0.14^a	0.18^a	0.12^a	0.07^b
	10 mg L^{-1}	66.32^a	68.51^a	46.11^a	50.19^a
	100 mg L^{-1}	763.89^a	745.68^a	500.53^a	579.17^a
4	0 mg L^{-1}	0.015^a	0.015^a	0.010^a	0.012^b
	0.02 mg L^{-1}	0.10^a	0.09^a	0.08^a	0.07^a
	10 mg L^{-1}	55.61^a	64.23^b	42.33^a	51.38^b
	100 mg L^{-1}	624.38^a	606.65^a	444.06^a	569.56^b

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removed. Mass balance calculations indicated that both *Tamarix* and *Salix* removed perchlorate from treatment solutions (Table 8). At the 0.02 mg L^{-1} exposure concentration, *Salix* removed 0.11 mg of perchlorate or 16% of the available perchlorate while *Tamarix* removed 0.02 mg (3%) of perchlorate. At the moderate exposure level of 10 mg L^{-1}, *Tamarix* removed slightly more mass (54.09 mg or 20% removed) than *Salix* (46.70 mg or 19% removed). *Salix* exposed to 100 mg L^{-1} of perchlorate removed 572.68 mg (22% removed) of perchlorate mass from solution. For the same exposure group, *Tamarix* only removed 414.43 mg (15% removed) of perchlorate from solution. Removal showed significant differences ($P < 0.05$) between species at the 0 mg L^{-1} treatment but not for any other treatment (Table 8). There was also a significant difference ($P < 0.05$) in removal between treatments, with all treatments different from each other except the *Tamarix* 0 mg L^{-1} and 0.02 mg L^{-1} treatments.

Perchlorate mass removed as a function of harvested biomass provides a useful measure of total phytoremediation per tank. These data standardize losses from the tank as a function of plant biomass. Possible mechanisms of loss include plant uptake and degradation/transformation. *Salix* tanks appear to remove more perchlorate than *Tamarix* at both high and low treatment concentrations; however, *Tamarix* accumulated more perchlorate at the moderate treatment (Table 9). For example, *Salix* tanks showed 8% more removal at the highest treatment and almost six fold more removal at the lowest treatment. *Tamarix* tanks, however, showed more than 69% more removal than *Salix* tanks at the moderate treatment level. Mass removed per harvested biomass also increased by an order of magnitude between the medium and high treatments, which is a likely effect since the treatments also differed by an order of magnitude. Differences

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Table 8. Perchlorate (mg) added to solution, removed from solution, and accumulated int*Salix exigua* and *Tamarix ramosissima* cuttings grown in hydroponic solution exposed to four ammonium perchlorate treatments. Different superscript letters indicate significant differences ($P < 0.05$) between species and treatments.

Treatment	Species	Perchlorate added (mg)	Perchlorate removed (mg)	Percent of perchlorte added removed	Perchlorate accumulated by plant (mg)	Percent of perchlorte added accumulated by plant
0 mg L^{-1}	*Salix exigua*	0.06^{a}	0.02^{a}	30%	1.94^{a}	-
	Tamarix ramosissima	0.06^{a}	0.01^{bf}	16%	3.26^{a}	-
0.02 mg L^{-1}	*Salix exigua*	0.68^{b}	0.11^{c}	16%	1.81^{a}	-
	Tamarix ramosissima	0.55^{b}	0.02^{cf}	3%	2.29^{a}	-
10 mg L^{-1}	*Salix exigua*	249.46^{c}	46.70^{d}	19%	26.48^{b}	11%
	Tamarix ramosissima	266.06^{c}	54.09^{d}	20%	34.65^{b}	13%
100 mg L^{-1}	*Salix exigua*	2580.28^{d}	572.68^{e}	22%	312.21^{c}	12%
	Tamarix ramosissima	2754.54^{e}	414.43^{e}	15%	265.00^{c}	10%

Table 9. Perchlorate removed from solution per harvested dry weight biomass (mg/kg) for *Salix exigua* and *Tamarix ramosissima* cuttings planted in hydroponic solution exposed to four ammonium perchlorate treatments. Different superscript letters indicate significant differences ($P < 0.05$) between species and treatments.

	Species	
Treatment	*Salix exigua*	*Tamarix ramosissima*
0 mg L^{-1}	0.14^{a}	0.13^{a}
0.02 mg L^{-1}	0.91^{a}	0.15^{a}
10 mg L^{-1}	410.53^{b}	695.16^{b}
100 mg L^{-1}	5003.49^{c}	4614.26^{c}

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between species for each treatment, however, were not significant ($P < 0.05$) (Table 9). Since removal of perchlorate mass as a function of harvested biomass shows no significant difference between species, it appears that the two species are equal in their potential for removing perchlorate from solution. These data have shown that under like conditions, both plants can remove perchlorate from the environment. Moreover, Urbansky et al. (2000) detected perchlorate in dormant stems from *Tamarix* growing in Las Vegas Wash, which along with these data suggests that removal would be expected at a field scale.

Perchlorate removal rates increased for both species and most treatment groups over the first 15 days (periods 1-3; Table 10). The 0.02 mg L^{-1} *Tamarix* treatment, however, showed a net increase in perchlorate mass in the tanks during the experiment and therefore removal rates were negative. Considering the low concentration of this treatment group, it is easily susceptible to evapoconcentration. The percent increase of perchlorate removed from period three to period four was not as high as it was during previous periods. *Tamarix* was observed to remove perchlorate more quickly than *Salix* in the 10 and 100 mg L^{-1} treatments; however, *Salix* was accumulating more mass than *Tamarix* during period three. *Salix* continued to accumulate more mass than *Tamarix* at the 100 mg L^{-1} concentration during period four.

Removal rates significantly differed ($P < 0.05$) between species at the 0 mg L^{-1} treatment but not for other species comparisons (Table 10). Species differences at the 0 mg L^{-1} treatment are indicative of ET and not removal differences, because mass balance calculations used one-half the method detection limit for all samples. For *Salix* in the 10 mg L^{-1} treatment, removal rates increased steadily for the first three periods but slowed

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Table 10. Total perchlorate removed (mg) and removal rates (mg day^{-1}) by *Salix exigua* and *Tamarix ramosissima* cuttings exposed to four ammonium perchlorate treatments for four consecutive 5-day periods. Different superscript letters indicate significant differences ($P < 0.001$) between species. One-half the method detection limit (0.004 mg L^{-1}) was used to construct mass removal for the 0 mg L^{-1} treatment.

		Salix exigua			*Tamarix ramosissima*		
Treatment	Period	Total perchlorate removed (mg)	Removal rates (mg day^{-1})	Percent increase from previous period	Total perchlorate removed (mg)	Removal rates (mg day^{-1})	Percent increase from previous period
0 mg L^{-1}	1	0.0029^{a}	0.0006^{a}	-	0.0016^{b}	0.0003^{b}	-
	2	0.0074^{a}	0.0007^{a}	60%	0.0040^{b}	0.0004^{b}	61%
	3	0.0137^{a}	0.0009^{a}	46%	0.0066^{b}	0.0004^{b}	39%
	4	0.0183^{a}	0.0009^{a}	25%	0.0098^{b}	0.0005^{b}	32%
0.02 mg L^{-1}	1	0.020^{a}	0.004^{a}	-	-0.059^{a}	-0.0012^{a}	-
	2	0.059^{a}	0.006^{a}	66%	-0.116^{a}	-0.0012^{a}	-
	3	0.083^{a}	0.006^{a}	28%	0.000^{a}	0.000^{a}	-
	4	0.107^{a}	0.005^{a}	23%	0.015^{a}	0.001^{a}	100%
10 mg L^{-1}	1	3.24^{a}	0.65^{a}	-	7.92^{a}	1.58^{a}	-
	2	13.21^{a}	1.32^{a}	75%	22.92^{a}	2.29^{a}	65%
	3	33.42^{a}	2.23^{a}	60%	41.24^{a}	2.75^{a}	44%
	4	46.70^{a}	2.34^{a}	28%	54.09^{a}	2.70^{a}	24%
100 mg L^{-1}	1	46.59^{a}	9.32^{a}	-	80.50^{a}	16.10^{a}	-
	2	129.00^{a}	12.90^{a}	64%	210.83^{a}	21.08^{a}	62%
	3	392.36^{a}	26.16^{a}	67%	377.35^{a}	25.16^{a}	44%
	4	572.68^{a}	28.63^{a}	31%	414.43^{a}	20.72^{a}	9%

down by period four. *Salix* in the 100 mg L^{-1} treatment showed similar results to the 10 mg L^{-1} treatment with steadily increasing removal rates followed by a slow down during period four. Removal rates for the *Salix* 100 mg L^{-1} treatment, however, did not slow down as much as the 10 mg L^{-1} treatment. Trends in removal by *Tamarix* were similar to the 10 and 100 mg L^{-1} *Salix* treatments, however, *Tamarix* removed perchlorate more quickly. At the 10 mg L^{-1} treatment, *Tamarix* had higher perchlorate removal rates then *Salix* for all periods, however, removal rates from period three to period four for *Tamarix* slowed down even more than *Salix* during the same time. The trends in removal rates show that there is a considerable capacity for both species to remove perchlorate once exposed. In the short term (10-day), it appears that *Tamarix* can remove more perchlorate than *Salix*. However, exposure extending past the first 10-day shows that *Salix* removed more perchlorate than *Tamarix* at the 100 mg L^{-1} group. It is not clear what level of removal would occur under prolonged exposure since this study only evaluated a 20-day period. Removal does not appear to be driven exclusively by ET in the tanks since ET increased linearly with time and removal was starting to slow down in the 100 mg L^{-1} *Tamarix* treatment by the last period (Figure 7). Plotting total perchlorate removed against ET for each period illustrates that removal begins to slow down under progressively higher ET (Figure 8).

Perchlorate Accumulation – Tissue Data

Solutions were extracted from various plant tissues and were directly analyzed for perchlorate by IC. These data evaluate the concentration and the corresponding mass of perchlorate that had accumulated in different parts of the plant. Since plants were

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Figure 7. Total perchlorate removed (mg) ± SE per period by *Salix exigua* (white bars) and *Tamarix ramosissima* (gray bars) for the a) 0 mg L^{-1}, b) 0.02 mg L^{-1}, c) 10 mg L^{-1}, and d) 100 mg L^{-1} ammonium perchlorate treatments. Different superscript letters indicate significant differences ($P < 0.05$).

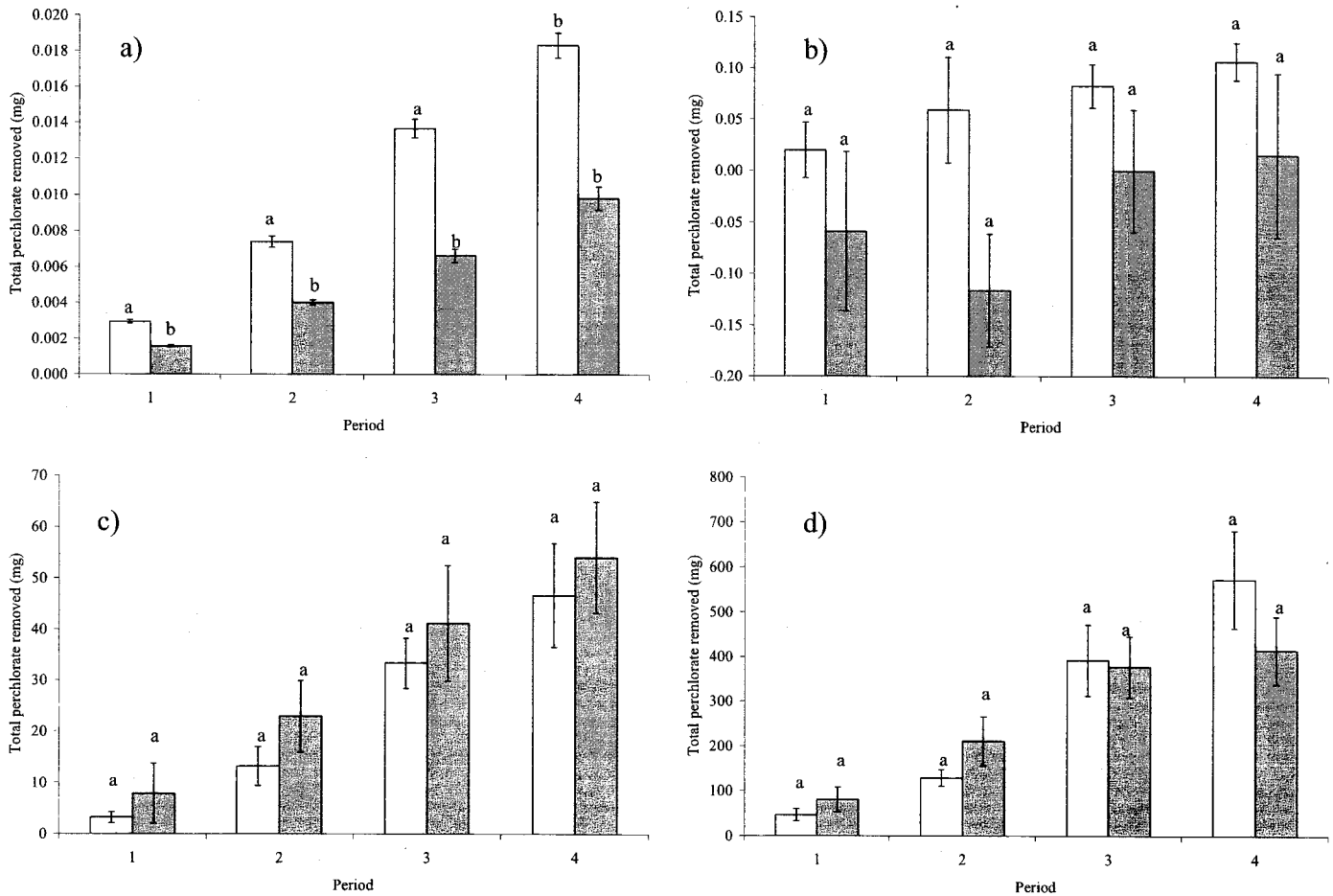

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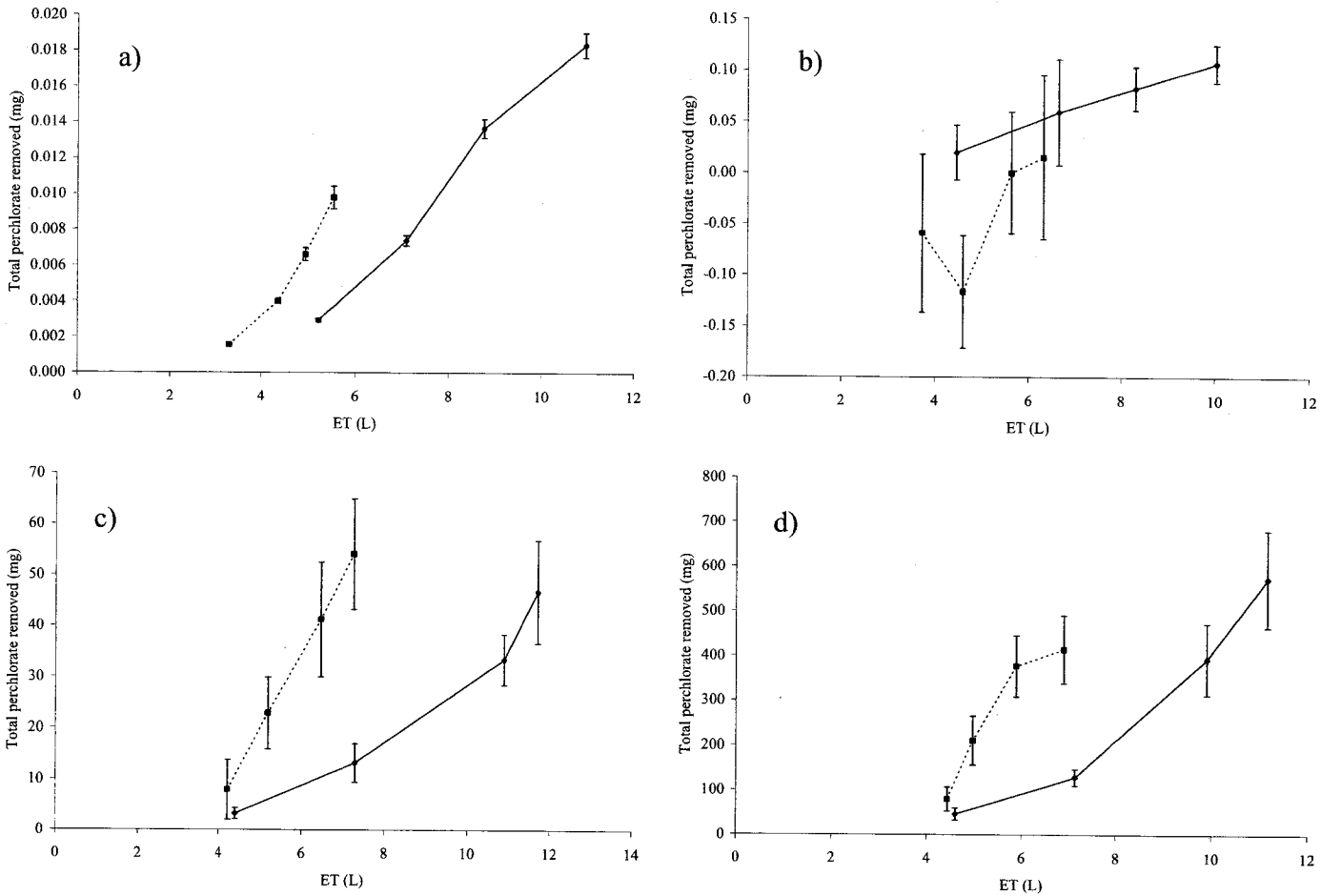
Figure 8. Total perchlorate removed (mg) ± SE per evapotranspiration (L) for each period. *Salix exigua* is the solid line and *Tamarix ramosissima* is the dashed line. Ammonium perchlorate treatments include a) 0 mg L^{-1}, b) 0.02 mg L^{-1}, c) 10 mg L^{-1}, and d) 100 mg L^{-1}.



harvested at the end of the 20-day exposure period, accumulation data represent cumulative perchlorate exposure for the length of the experiment. Leaf, stem, and root tissues, leaf exudate, and burned leaf material (ash) were all analyzed for perchlorate, which provides a detailed assessment of perchlorate storage and translocation within the plant. These data combined with the solution removal data described above, provide a more detailed assessment of the phytoremediation efficiency of *Salix* and *Tamarix*.

Total Perchlorate

Perchlorate concentrations in plant tissue (sum of perchlorate mass in plant tissue divided by total biomass) varied by species and treatment. The mean total concentration of perchlorate for all tissues combined showed a trend where plants exposed to higher treatments generally had higher concentrations of perchlorate in plant tissues (Table 11). This was most obvious for the highest two treatments, but less so for the lowest treatment and the control. Comparisons between species within all treatment groups showed significant differences ($P < 0.05$). *Salix* had higher mean concentrations of perchlorate than *Tamarix* at the 10 mg L^{-1} and 100 mg L^{-1} treatments. The magnitude of difference at these treatments shows that *Salix* concentrations were more than four times greater than *Tamarix*. Interestingly, tissue concentration data contrast sharply with removal data. Perchlorate mass removed from solution per unit of harvested biomass shows that *Salix* and *Tamarix* were not significantly different (Table 9), however, perchlorate detected in plant tissue appears to be significantly different (Table 11). At the moderate and high treatments, perchlorate removed from solution per unit of harvested biomass is higher then what is actually sequestered in the plants tissue. This seems to suggest that for the

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Table 11. Perchlorate concentration for all tissues combined (mg kg^{-1}). Different superscript letters indicate that log transformed data are significantly different ($P < 0.05$) between treatments and species.

	Treatment			
Species	0 mg L^{-1}	0.02 mg L^{-1}	10 mg L^{-1}	100 mg L^{-1}
Salix exigua	14.11^{a}	15.41^{a}	222.74^{b}	2615.22^{c}
Tamarix ramosissima	42.36^{bc}	27.34^{b}	56.68^{c}	487.93^{d}

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two highest concentration treatments, perchlorate is not only removed from solution but it may also be degraded or transformed to a non-perchlorate form. These data, however, do not include perchlorate that was exuded onto the surface of the leaf, which is a substantial component of accumulation (see below for discussion of exudate mass).

Treatment group comparisons within species showed subtle differences (Table 11). For *Salix*, significant differences ($P < 0.05$) were detected between all pairwise treatment comparisons except for the 0 mg L^{-1} and 0.02 mg L^{-1} treatments. *Tamarix* was significantly different ($P < 0.05$) between all pairwise treatment comparisons except for the 0 mg L^{-1} and 0.02 mg L^{-1} treatments and the 0 mg L^{-1} and 10 mg L^{-1} treatments. The greatest magnitude of difference was *Salix* at the 0 mg L^{-1} and 100 mg L^{-1} treatments with the latter being more than 185 times greater than the former. Since plants from the 0 mg L^{-1} treatment exhibited detectable concentrations of perchlorate but perchlorate was not detected in the solution, perchlorate must have already been in the plant when it was collected, was manufactured within the plant, or sub-detection perchlorate concentrations were present in the nutrient solution. For the latter to be true, *Salix* and *Tamarix* would have to transpire high volumes of solution.

To test if the perchlorate that was found in the tissue of the control group species was a result of solution derived perchlorate, a simple assessment of the minimum expected perchlorate concentration that would have to be present in the plants transpiration stream was calculated. For example, if 10 mg of perchlorate was found in *Salix* tissue and *Salix* transpired 100 L of solution, than it is reasonable to assume that the transpiration stream would have to have a minimum concentration of 0.1 mg L^{-1}. For this example to be true, *Salix* would have to retain within its tissue all of the perchlorate that is in its transpiration

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stream. Data from this study suggest that only 15 to 22% of the perchlorate that is in solution is actually removed and 10 to 13% is accumulated in plant tissue (Table 8). Therefore, the minimum concentration that would have to be in solution for a plant to accumulate 10 mg of perchlorate would be greater than the 0.1 mg L^{-1} calculated concentration. Although this evaluation method is a coarse assessment of the potential exposure from the solution pathway and it likely does not truly represent the complex nature of perchlorate tissue accumulation, it does provide a simple basis for assessment. Therefore, based on the control group data reported from this study and the calculation described above, the *Tamarix* transpiration stream solution would need to contain a minimum concentration of 0.18 mg L^{-1} and *Salix* would need to contain a minimum concentration of 0.06 mg L^{-1} (Table 12). Since both of these concentrations are above the MDL that was reported for the control solution and they are significantly different from each other ($P < 0.05$), it is highly unlikely that tissues were exposed to perchlorate from solution. Plants may have been exposed to perchlorate at the collection location, but the cuttings were not tested for this. It is possible for plants that are not exposed to a known contamination source to contain detectable levels of perchlorate. Observations by Snyder et al. (2005) and Plummer et al. (2006) indicate that perchlorate is commonly found in surface waters, which may likely be caused by atmospheric based processes (Dasgupta et al. 2005). Harvey et al. (2006) analyzed more than five different desert plants from remote locations in Oregon, Nevada, Arizona, and New Mexico and found perchlorate in their tissue. Creosote bush (*Larrea tridentata*), the most characteristic shrub found in the Mojave Desert, was found to have between 0.05 and 5.8 mg kg^{-1} of perchlorate (Harvey et al. 2006). Other plants tested and the amounts of perchlorate found in their tissues are

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Table 12. Calculated perchlorate concentration that would need to be in the transpiration stream of *Salix exigua* and *Tamarix ramosissima* cuttings to get the observed perchlorate mass that was accumulated in these plants at the 0 mg L^{-1} ammonium perchlorate treatment. Different superscript letters indicate significant differences ($P < 0.05$) between species.

Treatment	Species	Tank	Total ET (L)	Perchlorate accumulated (mg)	Perchlorate accumulated divided by total ET (mg L^{-1})	Perchlorate accumulated divided by total ET for all tanks (mg L^{-1})
0 mg L^{-1}	*Salix exigua*	19	29.53	2.20	0.07	0.06^{a}
		21	32.62	3.16	0.10	
		22	33.9	0.47	0.01	
	Tamarix ramosissima	1	17.72	3.48	0.20	0.18^{b}
		4	17.52	3.75	0.21	
		12	19.2	2.56	0.13	

white bursage (*Ambrosia dumosa*) at 0.36-0.9 mg kg^{-1}, sagebrush (*Artemisia tridentata*) 0.02-0.47 mg kg^{-1}, grasses 0.01-0.15 mg kg^{-1}, and cactus (*Opuntia* spp.) 0.07-3.2 mg kg^{-1}. These investigations suggest that the detectable concentrations of perchlorate found during the present study may derive from sources other than the treatment exposure.

Mass balance calculations that incorporate perchlorate exuded onto the surface of leaves show that *Tamarix* and *Salix* accumulated similar amounts of perchlorate. With exudate data factored in, at the 100 mg L^{-1} treatment, *Salix* accumulated a mean mass of 312 mg while *Tamarix* accumulated a slightly smaller amount of 265 mg (Table 8). Species differences in uptake at this concentration, however, were not significant ($P > 0.05$). At the 10 mg L^{-1}, 0.02 mg L^{-1}, and 0 mg L^{-1} treatments, *Tamarix* accumulated more perchlorate mass than *Salix*, however they were not significant different ($P > 0.05$). Perchlorate mass in plant tissue and exudate combined differed by more than seven fold from the 10 mg L^{-1} to 100 mg L^{-1} treatment. Mean perchlorate mass accumulated for the 0 mg L^{-1} treatment was higher than the 0.02 mg L^{-1} treatment. There was not a significant difference ($P > 0.05$) in mean perchlorate mass accumulated between the 0 mg L^{-1} and 0.02 mg L^{-1} treatments across species comparisons. Within species comparisons showed that accumulated mass differed between treatments, such that the 10 mg L^{-1} and 100 mg L^{-1} treatments were significantly different ($P < 0.05$) from each other and the other treatments (Table 8). The 0 mg L^{-1} and 0.02 mg L^{-1} treatments were not significantly different ($P > 0.05$) from each other.

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Leaf, Stem, and Root Perchlorate

Salix had the highest mean concentration of perchlorate in leaf, stem, and root material (referred to as matrices [plural] or matrix [single]) at the 10 mg L^{-1} and 100 mg L^{-1} treatments when compared to *Tamarix* tissues (Table 13). Average *Salix* leaf perchlorate concentrations were more than six fold greater than *Tamarix* leaves at the 10 mg L^{-1} and 100 mg L^{-1} treatments. Mean concentrations were greatest for plant tissue exposed to the 100 mg L^{-1} treatment and they generally declined with exposure. Concentrations for *Salix* and *Tamarix* at the 10 mg L^{-1} and 100 mg L^{-1} levels followed the pattern leaf > root > stem whereas for *Salix* at the control and 0.02 mg L^{-1} treatment it was leaf > stem > root. *Tamarix* at the 0.02 mg L^{-1} level followed a stem > root > leaf trend and at the control level it followed root > stem > leaf. Control plants had detectable concentrations of perchlorate and for some tissues and species these values surpassed concentrations found in some plants at the 10 mg L^{-1} treatment (Table 13). In most comparisons, control plants had greater concentrations than plants at the 0.02 mg L^{-1} treatment.

Species and treatment effects were detected for leaf samples (Table 13). Perchlorate found in leaf tissues were significantly different ($P < 0.05$) between species at the 10 mg L^{-1} and 100 mg L^{-1} treatments only, with *Salix* concentrating six fold more perchlorate in its leaves that *Tamarix*. Moreover, perchlorate concentrations at the leaves were generally the highest. Species effects were not detected at the lowest two treatments. Across treatments but within species, there was no difference ($P > 0.05$) in leaf perchlorate concentration between the 0 mg L^{-1} and 0.02 mg L^{-1} treatments, however, the

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Table 13. Perchlorate (mg kg^{-1}) detected in *Salix exigua* and *Tamarix ramosissima* leaf, stem, and root tissues on a dry weight basis that were exposed to four ammonium perchlorate treatments for four consecutive 5-day periods. Different superscript letters indicate significant differences ($P < 0.05$) between species, treatments, or matrices.

		Treatment			
Species	Matrix	0 mg L^{-1}	0.02 mg L^{-1}	10 mg L^{-1}	100 mg L^{-1}
Salix exigua	Leaf	35.04^{h}	42.59^{h}	559.68^{b}	6127.48^{c}
	Stem	6.28^{h}	3.26^{e}	56.08^{k}	499.52^{g}
	Root	1.40^{ehl}	1.80^{em}	70.45^{kn}	874.25^{g}
Tamarix ramosissima	Leaf	25.52^{hj}	12.95^{ah}	83.45^{d}	1005.82^{e}
	Stem	48.15^{ij}	39.94^{ai}	32.49^{dik}	78.43^{i}
	Root	54.45^{jl}	33.54^{ajm}	61.86^{djn}	298.69^{b}

10 mg L^{-1} and 100 mg L^{-1} treatments were significantly different ($P < 0.05$) from each other and the other treatments (Table 13).

Stem perchlorate concentration data showed differences between species and treatments. There were significant differences ($P < 0.05$) between species for all treatment groups except the 10 mg L^{-1} treatment (Table 13). *Salix* and *Tamarix* differed substantially in their accumulation of perchlorate in stem tissue. Within *Salix*, there was a significant difference ($P < 0.05$) in treatments for all pairwise comparisons (Table 13). However, there was not a difference ($P > 0.05$) in any pairwise comparisons for *Tamarix* stems across treatments. Since perchlorate concentrations in stems did not differ for *Tamarix* in any treatment, *Tamarix* does not appear to preferentially store perchlorate in stems under these experimental conditions. However, in the field with mature plants, a greater percentage of biomass would be associated with woody stems, therefore it is unknown if the results reported in this study would be observed. These data do suggest that stem based concentrations of perchlorate for *Tamarix* may not be good indicators of exposure. Urbansky et al. (2000), for example, may have underestimated perchlorate exposure in the Las Vegas Wash when only stems were sampled. Moreover, *Tamarix* stems may not be good sentinels for perchlorate contamination.

For root perchlorate concentration data, species comparisons were significantly different ($P < 0.05$) only at the 100 mg L^{-1} treatment (Table 13). Across treatments (Table 13), *Tamarix* was significantly different between the 100 mg L^{-1} treatment and other treatments. *Salix* revealed a similar response with the exception that the 10 mg L^{-1} and 100 mg L^{-1} treatments were significantly different ($P < 0.05$) than all other treatments and each other.

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Besides treatment and species effects, there were also plant tissue matrix effects (Table 13). At the 0 mg L^{-1}, 0.02 mg L^{-1}, and 10 mg L^{-1} treatments, there were no differences ($P > 0.05$) in matrix concentrations for *Tamarix* or for *Salix* at the 0 mg L^{-1} treatment. *Salix* leaf tissue at the 0.02 mg L^{-1} treatment and higher showed significant differences ($P < 0.05$) when compared to root or stem tissue. The only differences in *Tamarix* tissues were at the 100 mg L^{-1} treatment. All pairwise comparisons of tissue concentrations at this treatment were significantly different ($P < 0.05$). The data suggest that field level tissue sampling programs for either species should consider matrix differences and that low level exposure in the field is difficult to detect with matrix samples.

Ash Perchlorate

Perchlorate was not detected at a MDL of 0.05 mg L^{-1} in ashed leaf samples for either *Tamarix* or *Salix*. To evaluate potential differences in perchlorate in leaf ash, one-half the MDL was used for calculating purposes. Perchlorate concentration in ash shows a significant species effect, with *Tamarix* concentrations higher at all treatments (Table 14). Within species, no differences ($P > 0.05$) were detected across treatments. Perchlorate mass calculated from concentration data shows that there generally is not a treatment effect, except at the 100 mg L^{-1} treatment, with the mean mass values being significantly different ($P < 0.05$) from the other treatments. Since one-half the MDL was used to derive the ash perchlorate concentrations described above, this assessment likely does not truly represent actual species or treatment differences. The differences described here appear to be driven by biomass differences, because as the value of the

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Table 14. Perchlorate mass (mg) and concentration (mg kg^{-1}) detected in burned *Salix exigua* and *Tamarix ramosissima* leaf tissue (ash) grown hydroponically in four ammonium perchlorate treatments. Different superscript letters indicate significant differences ($P < 0.05$) between species or treatments.

		Species	
	Treatment	*Salix exigua*	*Tamarix ramosissima*
Perchlorate mass (mg)	0 mg L^{-1}	0.003^{a}	0.004^{ac}
	0.02 mg L^{-1}	0.003^{a}	0.003^{ac}
	10 mg L^{-1}	0.003^{a}	0.004^{ac}
	100 mg L^{-1}	0.003^{a}	0.004^{bc}
Perchlorate concentration (mg kg^{-1})	0 mg L^{-1}	0.22^{a}	0.34^{b}
	0.02 mg L^{-1}	0.23^{a}	0.39^{b}
	10 mg L^{-1}	0.21^{a}	0.36^{b}
	100 mg L^{-1}	0.24^{a}	0.32^{b}

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denominator (biomass) decreases (see Table 5), and the value of the numerator (one-half the MDL) remains constant, perchlorate ash concentration would increase. Interestingly, however, this assessment provides support for using a slash and burn phytoremediation method with either species. Recall that ashed samples originally consisted of leaf material and that leaf material was found to have the highest concentration of perchlorate. At the two highest treatments, more than 81% and 57% of the total perchlorate mass found in *Salix* and *Tamarix*, respectively, was found in the leaf (Table 15; exudate mass is discussed below and is not considered in this calculation). As such, phytoremediation programs that use *Salix* and *Tamarix* and include burning as a removal tool would be more successful than programs that do not include burning. Since *Tamarix* and *Salix* vigorously resprout after burning (Gary and Horton 1965; Rowe and Scotter 1973; Zasada 1986; Brotherson and Field 1987; Conrad 1987), burning would be a relatively simple management tool to implement. Species that resprout after burning have a greater advantage than species that grow from seed because the former has an intact, presumably extensive root system that could immediatly support the growing plants needs. For example, Busch and Smith (1995) reported that burning has contributed to the decline of native woody species in riparian areas because of the advantages of resprouting over seed establishment.

Exudate Perchlorate

Perchlorate exuded onto leaf surfaces separated statistically by treatments and species (Table 16). Perchlorate concentrations were standardized by leaf biomass so that direct comparisons could be evaluated between and within species. Significant differences ($P <$

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Table 15. Perchlorate accumulated (mg) in *Salix exigua* and *Tamarix ramosissima* leaf, stem, and root tissues exposed to four ammonium perchlorate treatments over a 20-day period. The percentage of perchlorate that was accumulated in the different tissue matrices from the total perchlorate accumulated into all tissue parts is listed in parentheses. These data do not include perchlorate that was depurated onto leaf surfaces.

		Perchlorate accumulated (mg)		
Treatment	Species	Root	Stem	Leaf
0 mg L^{-1}	*Salix exigua*	0.06 (3)	0.25 (13)	1.61 (83)
	Tamarix ramosissima	1.17 (36)	1.36 (42)	0.72 (22)
0.02 mg L^{-1}	*Salix exigua*	0.07 (4)	0.13 (7)	1.59 (88)
	Tamarix ramosissima	0.73 (33)	1.06 (48)	0.44 (20)
10 mg L^{-1}	*Salix exigua*	2.89 (11)	2.13 (8)	20.78 (81)
	Tamarix ramosissima	1.05 (23)	0.95 (20)	2.65 (57)
100 mg L^{-1}	*Salix exigua*	33.32 (11)	18.67 (6)	251.32 (83)
	Tamarix ramosissima	6.30 (15)	2.55 (6)	33.49 (79)

Table 16. Perchlorate mass (mg) and concentration based on a dry weight leaf mass basis (mg kg^{-1}) detected in *Salix exigua* and *Tamarix ramosissima* leaf exudate. Different superscript letters indicate significant differences ($P < 0.05$) between species or treatments.

		Treatment			
	Species	0 mg L^{-1}	0.02 mg L^{-1}	10 mg L^{-1}	100 mg L^{-1}
Perchlorate mass (mg)	*Salix exigua*	0.015^{a}	0.02^{a}	0.68^{b}	8.90^{c}
	Tamarix ramosissima	0.007^{a}	0.06^{b}	30.00^{c}	222.67^{d}
Perchlorate concentration (mg kg^{-1})	*Salix exigua*	0.34^{a}	0.46^{a}	19.12^{b}	216.89^{c}
	Tamarix ramosissima	0.26^{a}	1.9^{b}	979.24^{c}	6558.00^{d}

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0.05) in perchlorate concentrations on a mass basis were observed between species for all treatments except the 0 mg L^{-1} treatment. *Tamarix* generally had the greatest mean concentration of exudate with a maximum mean concentration of 6,558 mg kg^{-1} observed at the 100 mg L^{-1} treatment. The greatest difference between species was at the 10 mg L^{-1} treatment, with *Tamarix* having more than 50 times higher perchlorate concentration than *Salix*. Treatment differences were also observed with all *Tamarix* treatments being significantly different ($P < 0.05$) from each other. Pairwise comparisons of *Salix* show differences ($P < 0.05$) between treatments except for the 0 mg L^{-1} and 0.02 mg L^{-1} treatments. Perchlorate exudate mass results were similar to the concentration data results. Perchlorate mass exuded from leaf tissues revealed significant differences ($P < 0.05$) between species at all treatments except at the 0 mg L^{-1} level. Further, treatment differences were observed for all pairwise comparisons of *Tamarix* and *Salix* except for the lack of a difference observed for *Salix* at 0 mg L^{-1} and 0.02 mg L^{-1}.

Total mass exuded on to leaf surfaces was generally greatest for *Tamarix* (Table 16). At the 100 mg L^{-1} treatment, *Tamarix* exuded on average 222.7 mg of perchlorate while *Salix* only exuded 8.9 mg. A similar trend was observed at the 10 mg L^{-1} treatment level, where *Tamarix* exuded 30 mg of perchlorate compared to the 0.7 mg exuded by *Salix*. When exudate perchlorate mass was compared to the average perchlorate mass accumulated for all tissues, an interesting trend was detected. For each treatment level, *Salix* exuded less than 3% of the total perchlorate accumulated and *Tamarix* exuded through its leaves less than 3% of the total perchlorate accumulated at the 0 mg L^{-1} and 0.02 mg L^{-1} treatments (Table 17). *Tamarix*, however, exuded nearly 87% of the perchlorate accumulated at the 10 mg L^{-1} treatment and more than 84% of perchlorate

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Table 17. Perchlorate (mg) in *Salix exigua* and *Tamarix ramosissima* leaf, stem, and root tissues, exuded onto leaf surfaces, remaining in ash after leaves were burned, and total accumulated in all tissues combined (with and without exudate mass). Plants were exposed to four ammonium perchlorate treatments over a 20-day period. The percentage of perchlorate that was accumulated in the different tissue matrices from the total perchlorate accumulated into all tissue parts including exuded perchlorate is listed in parentheses.

		Perchlorate (mg)						
Treatment	Species	Root	Stem	Leaf	Exudate	Ash	Total (wihout exudate)	Total (with exudate)
0 mg L^{-1}	*Salix exigua*	0.06 (3)	0.25 (13)	1.61 (83)	0.01 (1)	0.0027 (<1)	1.93 (99)	19.4 (100)
	Tamarix ramosissima	1.17 (36)	1.36 (42)	0.72 (22)	0.01 (<1)	0.0042 (<1)	3.25 (99)	3.26 (100)
0.02 mg L^{-1}	*Salix exigua*	0.07 (4)	0.13 (7)	1.59 (87)	0.02 (1)	0.0026 (<1)	1.8 (99)	1.81 (100)
	Tamarix ramosissima	0.73 (32)	1.06 (46)	0.44 (19)	0.06 (3)	0.0032 (<1)	2.23 (97)	2.29 (100)
10 mg L^{-1}	*Salix exigua*	2.89 (11)	2.13 (8)	20.78 (78)	0.68 (3)	0.0026 (<1)	25.8 (97)	26.48 (100)
	Tamarix ramosissima	1.05 (3)	0.95 (3)	2.65 (8)	30 (87)	0.0036 (<1)	4.65 (13)	34.65 (100)
100 mg L^{-1}	*Salix exigua*	33.32 (11)	18.67 (6)	251.32 (80)	8.9 (3)	0.0028 (<1)	303.31 (97)	312.21 (100)
	Tamarix ramosissima	6.30 (2)	2.55 (1)	33.49 (13)	222.67 (84)	0.0039 (<1)	42.34 (16)	265 (100)

accumulated at the 100 mg L^{-1} treatment. The 0 mg L^{-1} and 0.02 mg L^{-1} treatments showed minimal exudate mass, with a more uniform distribution of perchlorate in stems, roots, and leaves.

Exudate data contrast with leaf data for both species. Leaf data show that *Salix* accumulates perchlorate in the leaf and exudate data show that *Tamarix* exudes perchlorate onto the leaf surface. These differences are most obvious for the two highest treatments. For example, more than 84% of the perchlorate that is taken up by *Tamarix* is exuded onto leaf surfaces at these treatments. For the same treatments, more than 78% of the perchlorate that is taken up by *Salix* is bound up within leaf tissue. Because there are high concentrations at and on the leaf, and since both species are deciduous, this is an important potential pathway for perchlorate cycling back into the environment.

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CHAPTER 5

RECOMMENDATIONS AND IMPLICATIONS FOR WATER RESOURCE MANAGEMENT

Perchlorate contamination in Southern Nevada has been found to originate from two former production facilities located near Henderson, Nevada. The most extensive source of perchlorate comes from a facility presently operated by Tronox Incorporated (Tronox), formerly known as Kerr-McGee Chemical, LLC a subsidiary of Kerr-McGee Corporation. Tronox discontinued commercial production of ammonium perchlorate in 1998 soon after perchlorate was detected in the Colorado River below Hoover Dam. As of September 2005, Tronox has spent $122,000,000 remediating perchlorate contamination at their Henderson facility (Tronox 2006). The perchlorate remediation strategy consisted of using three ion exchange units located near the Las Vegas Wash, which were fully operational by November 1999. By the fall of 2005, the ion exchange units were decommissioned and replaced by biologically based fluidized bed reactors (U.S. Environmental Protection Agency 2006). Treatment has been extremely effective considering that initial perchlorate loading in 1999 was approximately 408-454 kg day^{-1}. Perchlorate concentrations in the Las Vegas Wash, Lake Mead, and the lower Colorado River have decreased by 85%, 70%, and 60%, respectively (U.S. Environmental Protection Agency 2006). Treatment using these remediation strategies has, however, cost Tronox approximately $58,000 day^{-1} ($122,000,000 total cost divided by 2,099 days

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between January 2000 and September 2005). Considering the high cost of remediating perchlorate from groundwater near the Las Vegas Wash, phytoremediation may be a useful low cost alternative to these traditionally expensive techniques.

Both *Salix* and *Tamarix* were found to remove perchlorate from hydroponic solution and accumulate it into different tissue parts. *Salix* accumulated perchlorate primarily into its leaves while *Tamarix* exuded most of the perchlorate that it accumulated onto the surface of its leaves. Since both species are deciduous, leaves that contain perchlorate would be dropped in the fall and would be a source of perchlorate to cycle back into the environment. Because of this, using these species for phytoremediation is somewhat problematic. There are, however, important differences to consider. For example, between 84 and 87% of the perchlorate mass added to the highest two treatments was exuded onto the surface of *Tamarix* leaves, which highlights the fact that *Tamarix* is primarily a flow through system. Moreover, the perchlorate that is exuded is in a more soluble state than the perchlorate that is bound up into *Salix* leaf tissues. Because perchlorate salts are extremely soluble (Schumacher 1960), a minor rain event may provide enough of a rinsing effect to translocate perchlorate from the surface of the leaf. Once in soluble mobile state, perchlorate may migrate directly into surface water bodies or simply infiltrate into groundwater, which along the Las Vegas Wash would ultimately end up in Lake Mead. Leaves that have fallen and accumulated below *Tamarix* plants would also be rinsed of perchlorate during rain events. Therefore, any phytoremediation program using *Tamarix* would likely be unsuccessful. Because *Tamarix* is primarily a flow through system it would provide a minimal tangible benefits for cleaning up perchlorate in the environment. This is also important to consider since *Tamarix* is the

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dominant plant found along the Las Vegas Wash. Approximately 15.5 hectares of *Tamarix* already grow in areas of shallow groundwater that lie within the 10 mg L^{-1} perchlorate concentration contour as reported by Tronox (i.e., Kerr-McGee) in a 2004 monitoring report to the Nevada Division of Environmental Protection (Figure 9). Based on the experimental results of this study, it is reasonable to suggest that *Tamarix* growing in these areas is accumulating perchlorate but that it is exuding most of that perchlorate onto leaf surfaces. These plants, therefore, are providing minimal if any clean up of perchlorate near the Las Vegas Wash.

Salix is a better candidate than *Tamarix* for phytoremediation, but since *Salix* accumulates perchlorate primarily into its leaves, it too is problematic. Approximately 78 to 80% of the perchlorate mass accumulated by *Salix* at the two highest treatments was found in leaf tissue (Table 17). *Salix*, like *Tamarix*, is deciduous, which suggests that perchlorate bound up in leaf tissue would be returned to the environment as a result of leaf fall. Since leaf bound perchlorate would not be readily assimilated back into the environment, downed leaf material would likely be releasing perchlorate slowly over time as the leaves decomposed. Pomeroy et al. (2000) described the decomposition rate of *Salix* leaf material in the Colorado River and showed that after 142 days, 52% of the leaf mass remained. *Salix* leaves were also shown to decompose more slowly than *Tamarix* leaves. Along the Las Vegas Wash, *Salix* would likely have similar breakdown rates. It is uncertain, however, when perchlorate would be mobilized from the leaves and if the biochemical processes that contribute to leaf breakdown would provide for perchlorate degradation. Further, since Pomeroy et al. (2000) evaluated decomposition for submerged leaf packs, it is uncertain what decomposition rates would be expected for

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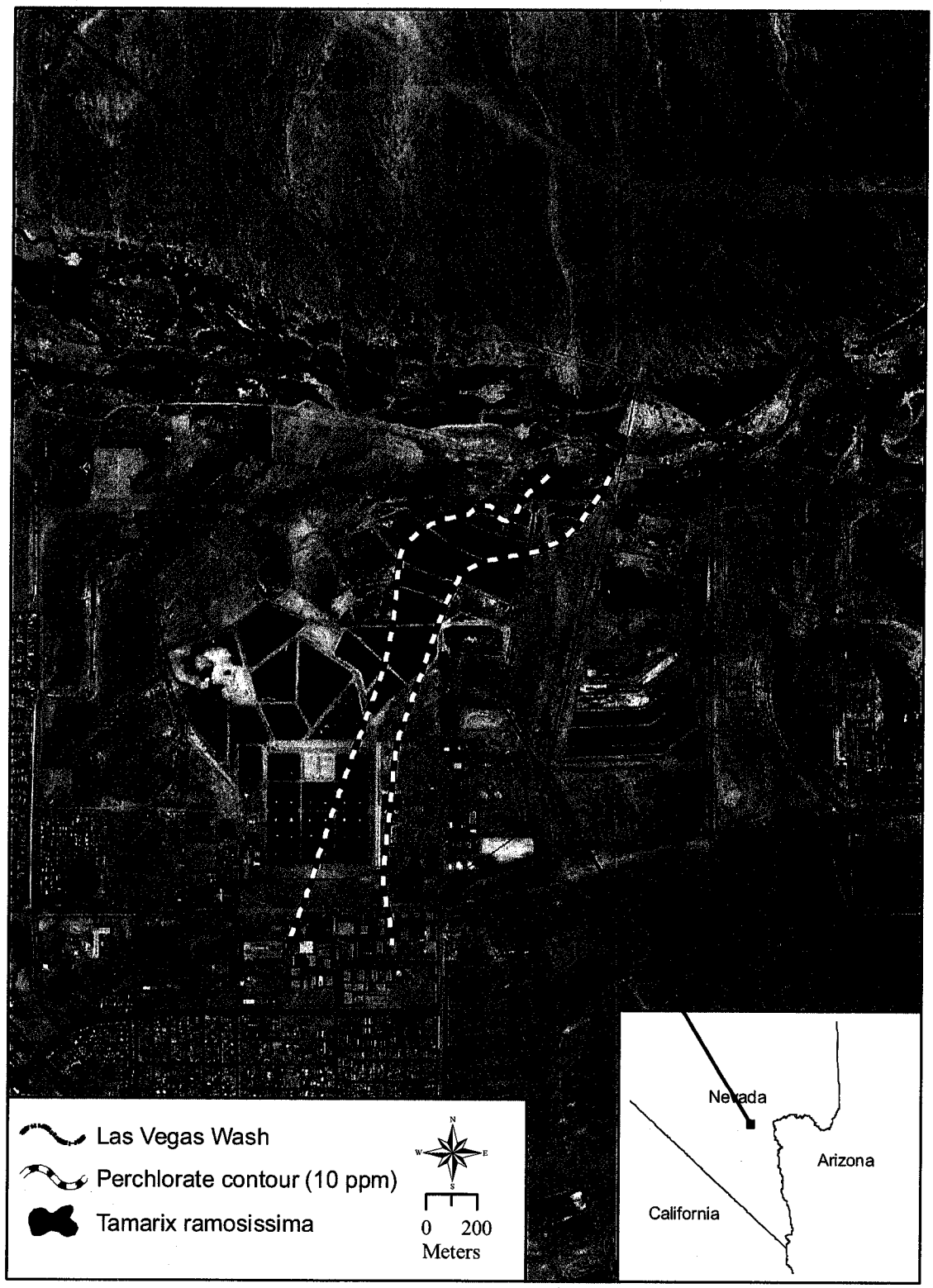
Figure 9. Extent of *Tamarix ramosissima* in the 10 mg L^{-1} shallow groundwater perchlorate plume adjacent to the Las Vegas Wash.



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terrestrial decomposition. Considering the abundance, diversity, and specialization of aquatic macroinvertebrates as decomposers, however, it is reasonable to suggest that terrestrial decomposition along an arid stream channel like the Las Vegas Wash would require a longer period of time than what was reported by Pomeroy et al. (2000). *Salix* growing away from the stream channel, therefore, would provide a greater benefit for remediation since leaf fall would not be as problematic. Because *Salix* tends to be more obligate than facultative in its use of groundwater (Cleverly et al. 1997), its utility for remediation would likely be limited to off channel irrigated areas. In or near channel plants would provide minimal benefit for cleaning up perchlorate unless a harvest strategy was employed.

Salix leaf material that was converted to ash by burning contained on average 0.003 mg of perchlorate for the highest treatment exposure. Since the mean leaf perchlorate mass for this same group was 251 mg, burning resulted in a 99.99% reduction of perchlorate from the leaf. Using *Salix* to accumulate perchlorate from contaminated sites and burning the plants prior to leaf fall would provide the greatest remediation benefit. Burning would also be a cheaper alternative to annually harvesting biomass. Unfortunately, having a controlled burn near the Las Vegas Wash is difficult because of its proximity to urban areas. Even controlled burning of slash material conducted as part of restoration initiatives along the Las Vegas Wash has gotten more difficult because of encroaching urban areas (pers. obs.). An additional justification for using *Salix* for phytoremediation would be the added benefit of creating natural riparian habitats along the Las Vegas Wash. This would help meet the goals of the multi-stakeholder

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collaborative Las Vegas Wash Coordination Committee which is already conducting an extensive native revegetation program along the Las Vegas Wash.

Perchlorate removed from solution as a function of biomass did not show significant differences between species (Table 9), however, perchlorate accumulated in plant tissue did show a significant difference between species (Table 11). With regards to *Tamarix*, the mass of perchlorate that was exuded onto the surface of its leaves was high enough to observe significant differences in accumulation between species. Accumulation data (unless specified), therefore, do not account for perchlorate that is not within the plant. To evaluate the feasibility of using these species for phytoremediation and to evaluate the phytoremediation that may already be occurring in *Tamarix* dominated areas along the Las Vegas Wash, plant removal rates and accumulation rates should be compared to the removal rates exhibited by the existing treatment strategy used by Tronox. For the purpose described above, accumulation rates for *Tamarix* should include perchlorate that is on leaf surfaces because any effective remediation strategy with this plant would require removing biomass before leaf fall. Also, considering that the shallow groundwater perchlorate concentration on Tronox property above their slurry wall is 1,200-1,500 mg L^{-1} and below the wall the concentration is 130-180 mg L^{-1} (U.S. Environmental Protection Agency 2006), the discussion provided herein is limited to the results from the highest two treatments.

Perchlorate removed per total harvested biomass per days of exposure (i.e., 20-day) for *Salix* tanks at the highest two treatments was 20.5 mg kg^{-1} day^{-1} for the 10 mg L^{-1} treatment and 250.2 mg kg^{-1} day^{-1} for the 100 mg L^{-1} treatment. For *Tamarix* tanks, the removal rate at the 10 mg L^{-1} treatment was 34.8 mg kg^{-1} day^{-1} and at the 100 mg L^{-1}

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treatment it was 230.7 mg kg^{-1} day^{-1}. Mean accumulation rates as a function of total harvested biomass and days of exposure were substantially less than the values reported above. *Salix* at the 10 mg L^{-1} and 100 mg L^{-1} treatments were 11.4 mg kg^{-1} day^{-1} and 134.6 mg kg^{-1} day^{-1}, respectively, and *Tamarix* at the 10 mg L^{-1} and 100 mg L^{-1} treatments were 21.8 mg kg^{-1} day^{-1} and 150.3 mg kg^{-1} day^{-1}, respectively. The removal rates indicated above represent the maximum rate that perchlorate could be removed from solution at a specific treatment concentration, whereas accumulation rates are a reflection of the minimum perchlorate that could be removed from solution and retained by the plant. This has important implications for remediation managers because it provides a useful measure of remediation efficiency. For phytoremediation to be considered an equal substitute to the current treatment process, however, these plants would need to remove approximately 771-907 kg day^{-1} or 271-331 metric tons $year^{-1}$, the current removal rate (U.S. Environmental Protection Agency 2006).

Several assumptions must first be stated to calculate an estimate of remediation that would rival Tronox's current results and to calculate the remediation that is likely already being provided by *Tamarix* in the field (Figure 9). These assumptions are that the trends reported in this study would be observed at a field scale, the annual growing season in Southern Nevada is approximately 259 days (i.e., the number of frost free days), the annually harvestable above ground biomass is 10.5-14.1 metric tons ha^{-1} (Meers et al. 2003), and 15.5 ha of land with a constant available groundwater concentration of 10 mg L^{-1} (Figure 9) is available to use as a phytoremediation plantation. The calculation used for these estimates is provided (Table 18). From these assumptions and based on the 10 mg L^{-1} data, the removal of perchlorate by *Salix* would be 0.86-1.15 metric tons $year^{-1}$ as

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Table 18. Formulas for calculating the minimum and maximum metric tons of perchlorate potentially removed annually. Metric tons of biomass produced is based on data from Meers et al. (2003).

Minimum

$$\frac{\textbf{X}\text{ mg of perchlorate removed}}{1\text{ kg of biomass produced}} \times \frac{}{1\text{ days of exposure}} \times \frac{257\text{ days of exposure}}{1\text{ year}} \times \frac{\textbf{10.5}\text{ metric tons of biomass produced (annually)}}{1\text{ hectare}} \times \frac{15.5\text{ hectare of land available for phytoremediation}}{} \times \frac{1000\text{ kg of biomass produced}}{1\text{ metric ton of biomass produced}} \times \frac{1\text{ metric ton of perchlorate removed}}{1{,}000{,}000{,}000\text{ mg of perchlorate removed}} = \frac{\textbf{X}\text{ metric tons of perchlorate removed}}{\text{year}}$$

Maximum

$$\frac{\textbf{X}\text{ mg of perchlorate removed}}{1\text{ kg of biomass produced}} \times \frac{}{1\text{ days of exposure}} \times \frac{257\text{ days of exposure}}{1\text{ year}} \times \frac{\textbf{14.1}\text{ metric tons of biomass produced (annually)}}{1\text{ hectare}} \times \frac{15.5\text{ hectare of land available for phytoremediation}}{} \times \frac{1000\text{ kg of biomass produced}}{1\text{ metric ton of biomass produced}} \times \frac{1\text{ metric ton of perchlorate removed}}{1{,}000{,}000{,}000\text{ mg of perchlorate removed}} = \frac{\textbf{X}\text{ metric tons of perchlorate removed}}{\text{year}}$$

calculated from removal data and 0.48-0.64 metric tons year^{-1} for accumulation data (Table 19). From these calculations, it is clear that *Tamarix* and *Salix* would provide less than 0.25% of the remediation needed to rival the current Tronox strategy. This simple calculation obviously highlights the fact that phytoremediation does not provide equal removal at a treatment exposure of 10 mg L^{-1}. The concentration of perchlorate in shallow groundwater flowing from the Tronox facility to the Las Vegas Wash, however, is substantially greater than 10 mg L^{-1} and if a plantation were established on a 100 mg L^{-1} location, the effects of phytoremediation increase substantially (Table 19). Phytoremediation would still only contribute slightly more than 1% to the current remediation. Higher concentrations of perchlorate in shallow groundwater would likely result in more remediation.

Although the assessment provided above offers insights towards implementing phytoremediation, this experiment only evaluated the uptake of perchlorate from hydroponic solution and therefore it is unknown exactly how much perchlorate would be removed at the field scale. Phytoremediation is, however, a reasonable remediation alternative that may be appropriate at lesser-contaminated sites or for sites where minimal resources can be directed. Moreover, these species can be used as sentinels of perchlorate contamination in riparian areas throughout the western U.S. Burning the above ground biomass that is high in perchlorate would be the most effort required by a remediation manager. A carefully evaluated cost-benefit approach is necessary for establishing remediation alternatives, including phytoremediation, at perchlorate contaminated sites.

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Table 19. Range of perchlorate removed annually from 15.5 ha of available land within the 10 mg L^{-1} perchlorate plume contour near the Las Vegas Wash.

			Perchlorate removed (metric tons ha^{-1} $year^{-1}$)			
			Derived from removal rate data		Derived from accumulation rate data	
Shallow groundwater concentration	Treatment	Range	*Salix exigua*	*Tamarix ramosissima*	*Salix exigua*	*Tamarix ramosissima*
10 mg L^{-1}	10 mg L^{-1}	Minimum	0.86	1.45	0.48	0.91
		Maximum	1.15	1.95	0.64	1.23
100 mg L^{-1}	100 mg L^{-1}	Minimum	10.46	9.65	5.63	6.09
		Maximum	14.05	12.96	7.56	8.17

Perchlorate contamination originating from the Tronox facility is being effectively remediated with the current method (U.S. Environmental Protection Agency 2006), albeit at considerable expense. Phytoremediation alternatives at this contaminated site would add little benefit to the current techniques. Phytoremediation, however, is an alternative treatment strategy which could be used when funding is limited and it may in some situations be the only feasible alternative. *Tamarix* does not appear to be a desirable species for phytoremediation because it is primarily a flow through system. Any benefit garnered by *Tamarix* accumulating perchlorate would be presumably lost with the first rain event or during leaf fall. Further, *Tamarix* is an invasive exotic plant that aggressively competes with and often displaces native woody plants (Busch and Smith 1995). The resulting landscape usually consists of a monoculture, which provides sub-optimal habitat for riparian dependent organisms. *Salix* is a superior alternative to *Tamarix* for phytoremediation because it accumulates and removes a similar amount of perchlorate as *Tamarix,* but the perchlorate that is accumulated is not readily cycled back into the environment. *Salix* accumulates perchlorate mostly in leaf tissues, which if burned have nearly no remaining perchlorate. In addition, *Salix* is a native species found in many western riparian areas and if planted provides the value added benefit of habitat improvement.

This study compared the removal of perchlorate from hydroponic solution by *Salix* and *Tamarix* and evaluated where perchlorate was accumulated. It is unknown if a field scale phytoremediation study using either species would exhibit similar results. Additional research should be conducted to determine if *Salix* and *Tamarix* accumulate perchlorate under field conditions. Moreover, research should be conducted to determine

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if perchlorate is accumulated in roots, stems, and leaves in the same proportions that were observed in this study. Most importantly, field studies should investigate the perchlorate exudation behavior of *Tamarix* under various groundwater perchlorate concentrations and what happens with perchlorate during leaf decomposition or burning. The feasibility of improving water quality with phytoremediation techniques at a field scale should be evaluated so that water resource managers can make informed decisions based on rigorous scientific data.

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