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RESPONSE OF TWO DESERT SHRUBS TO NITRATE

By

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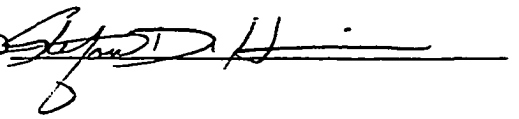
SIGNED 

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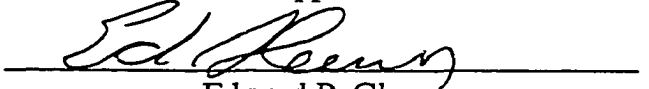 Nov 13 1998

Edward P. Glenn — Date

Professor of Soil, Water & Environmental Science

DEDICATION

For the Ones I Love

ACKNOWLEDGEMENTS

I dedicate this thesis to the three most influential people in my life, my husband, my mom, and my dad. My husband, Dean C. Hines, Ph.D, has provided me with incredible support on a daily basis. He has persevered through my career change and completion of my second masters degree (look out for the Ph.D) and he has helped shape me as a scientist. Dean has also continually shared with me his positive, loving spirit and expressed to me an unshakable faith in my abilities. Dean, you have helped make it happen; Thank you with all I am.

My mom, Linda Ashton, is a mother in the greatest sense of the word. She continually provides positive encouragement and never doubts my decisions (except when I was in high school, of course). She and I have a spiritual connection and friendship for which I am eternally grateful. Through her actions my mom has taught me perseverance and that you can overcome whatever obstacles life presents. Mom, you have broken the chain, thank you.

It was not until recently that I truly realized the influence my father, Wade R. Eichhorn, has had on me. He is a physicist who has a passion for astronomy; I married an astrophysicist. He tried working in the field of education and left with frustration; I worked in Native American education and left for a greater passion (this degree). He was the first person to introduce me to science and, upon reflection, it is not surprising I ended up in soil science. I remember him helping me put together my 7th grade science fair project "The Geology of Central Texas," for which I won second prize. When we would go for walks, my dad would always point out interesting plants and talk about the biology of the ecosystem. Well, my undergraduate minor was in biology and this research has a strong emphasis on plants. We also have many similarities in our personalities – energetic, an appreciation for travel, and love of the outdoors. Dad, I am truly proud to be my father's daughter.

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ABSTRACT

The response of two native Arizona plants, fourwing saltbush (*Atriplex canescens*) and greasewood (*Sarcobatus vermiculatus*), to five concentrations of nitrate (tap water only, 50 mg/L, 100 mg/L. 750 mg/L, and 2000 mg/L as nitrate) is investigated. Their growth, transpiration, and nitrate and percent nitrogen tissue concentrations were measured. All of the plants' responses were affected by nitrate concentration. In general, it can be concluded that both fourwing saltbush and greasewood tolerated nitrate concentrations as high as 2000 mg/L. However, greasewood's optimal growth was at Level 4 (750 mg/L nitrate) and its tissue nitrate approximately doubled from an average of 572 ± 255 mg/kg at Level 4 to 1020 ± 511 mg/kg at Level 5 (2000 mg/L nitrate). Fourwing saltbush demonstrated a remarkable ability to tolerate large quantities of nitrate and convert it to organic nitrogen at high concentrations. Fourwing saltbush's largest dry mass, 14.48 ± 2.03 g, was at 2000 mg/L of nitrate.

CHAPTER 1

INTRODUCTION

In this thesis I present original research investigating the response of two native Arizona plants, fourwing saltbush (*Atriplex canescens*) and greasewood (*Sarcobatus vermiculatus*), to five concentrations of nitrate (tap water only, 50 mg/L, 100 mg/L. 750 mg/L, and 2000 mg/L as nitrate). The purpose of the study was to determine if the plants could tolerate high nitrate concentrations (up to 2000 mg/L) and to quantify each plant's potential to remove nitrate. The impetus of the study was a proposal to use nitrate contaminated groundwater to aid in establishing native plants to revegetate a Uranium Mill Tailings Remedial Action (UMTRA) site in Monument Valley, Arizona. The plants' potential to remove nitrate is important to determine whether enough nitrate could be removed to prevent nitrate accumulation in the soil.

Chapter 1 provides background information on the UMTRA site and provides a general overview of nitrate sources and methods of nitrate remediation. Chapter 2 is a review of the nitrogen cycle and physiological responses of plants, especially *Atriplex* species, to nitrate. In Chapter 3, I present the experimental design and in Chapter 4 the experimental results. Chapter 5 is a discussion of the results and Chapter 6 presents conclusions and suggestions for further research.

1.1 Nitrate Sources

Geochemists estimate that the earth contains approximately 57.4 x 10^{18} kg of nitrogen, 93% of which is tied up in rocks with most of the remaining 7% occurring in the atmosphere primarily as N_2 (Lewis 1986). Approximately, 0.0025% (1.5 x 10^{15} kg) of the earth's total nitrogen is available to plants, and a little over half that amount is immediately available to plants in the form of the inorganic molecules nitrate (NO_3^-) and ammonium (NH_4^+) (Lewis 1986). Ammonium can be volatilized, adsorbed to soil particles, or converted to nitrate (Lewis 1986).

Nitrogen is an essential element for plants and is often the most limiting factor for plant growth (Fowden 1979). When an abundant supply of nitrogen is available, plant growth increases substantially (Stulen et al. 1990), which is why nitrogen-based fertilizers are applied to crops.

Most nitrate found in soil and groundwater is a result of natural inputs and agriculture (Miller and Donahue 1995). Nitrate can occur in localized regions in very concentrated amounts as a result of natural accumulation over time and from industries releasing nitrogen-based products, such as nitric acid or ammonia, into the environment. This is the case for many uranium milling and ore processing sites, like the one presented in this study, where large quantities of ammonia were used to neutralize acidic uranium/vanadium leachate batches. The result can be soil and groundwater heavily contaminated with nitrate and ammonia. With time, the ammonia is converted to nitrate by soil bacteria, making nitrate the most abundant contaminant.

Though nitrate is an important plant nutrient, high concentrations can be toxic to infants and animals. This is because, in the body, the nitrate is reduced to nitrite which causes methaemoglobinaemia and prevents oxygen transport (Mengel 1992). For this reason, it is important to remediate sites heavily contaminated with nitrate.

1.2 The Monument Valley UMTRA Site

The Monument Valley Uranium Mill Tailings Remedial Action (UMTRA) site (here after also referred to as the Site) is located in northern Arizona just south of the Utah border in the Monument Valley. Uranium and vanadium ore was mined from the Site beginning in 1943 and chemical processing of the ore occurred on-site between 1964 and 1968, resulting in sulfate, nitrate, calcium, ammonium, and trace metal contamination. Subsequent uranium decontamination efforts resulted in the loss of vegetation and topsoil from large regions of the Site.

The groundwater at this location contains a range of nitrate concentrations, from 44 mg/L to greater than 1000 mg/L as nitrate. In comparison, the U.S. Environmental Protection Agency has a drinking water limit for nitrate of 44 mg/L.

1.2.1 Site Specific Remediation Goals

There are several goals for remediation of the Monument Valley UMTRA site. The first is to revegetate the area because uranium decontamination efforts resulted in the loss of vegetation and topsoil from large regions of the Site. Other goals include removing the nitrate and ammonia from the soil and preventing continued migration of the nitrate

contaminated groundwater plume. Eventually, the groundwater may also be remediated directly.

When developing the revegetation plan for the Monument Valley UMTRA site we discussed whether or not the plants should be irrigated. Research into using fourwing saltbush for revegetation gave a strong indication that irrigation significantly increases revegetation success (Aldon 1972). Thus, it was decided that irrigation would be used to establish the plants. However, we debated about whether to irrigate with the nitrate contaminated groundwater located directly beneath the site, to use uncontaminated groundwater, or to use a blend. We decided that if the plants could tolerate the high nitrate concentrations, it would be better to irrigate with the contaminated groundwater. This would utilize otherwise unusable water and would preserve the high quality water for regional residents.

A potential problem with irrigating with nitrate contaminated water is that if the plants do not absorb the nitrate rapidly enough, the goal to remediate the soil may not be achieved. On the other hand, if the plants can extract large enough quantities of nitrate from the soil solution, and nitrate can be lost through denitrification rather than leaching, then soil remediation may be achieved. Also, if the water that migrates back down into the groundwater contains less nitrate than it had when it was initially extracted, at least partial remediation of the groundwater will be accomplished.

1.3 Methods of Nitrate Remediation

1.3.1 Removal of Hazardous Waste

Remediation of hazardous waste sites has traditionally been accomplished by physical and chemical technologies, such as activated carbon adsorption (Talley and Sleeper 1997). However, bioremediation, the use of biological activity to treat toxic waste, is becoming more widely used. Bioremediation mostly refers to the use of microbes for remediation, however plants are emerging as useful remediation tools as well, which is sometimes referred to as phytoremediation.

Remediation of contaminated soils can be *in situ* or *ex situ* . *In situ* treatment takes place on-site without excavation while *ex situ* treatment involves removing the soil or water and treating it elsewhere (Talley and Sleeper 1997). The selection of a treatment process depends on many factors, including specific site and contaminant considerations, cost, time scale, and extent of contaminant removal needed.

1.3.2 Traditional Nitrate Treatment Methods

Nitrate treatment methods mostly focus on removing nitrate from groundwater or public water supplies, rather than removing nitrate from soil. This is probably because nitrate

moves rapidly through the soil profile into water supplies, and water is the medium through which humans and animals can ingest toxic nitrate levels.

Ion exchange and biological denitrification are the two primary techniques used to remove nitrate from water (Solt 1986). Ion exchange is an *ex situ* method where nitrate is bound to a resin and an anion, such as chloride, replaces the nitrate. Limitations to the method include cost, disposal of nitrate-laden salts, high concentration of chloride and reduced efficiency in high sulfate water (Solt 1986).

Biological denitrification can be either *in situ* or*ex situ* and takes advantage of the conversion of nitrate to dinitrogen gas by bacteria. Denitrification is generally stimulated by anaerobic conditions, however some bacteria, such as *Thiosphaera pantotropha*, can denitrify under aerobic conditions (Kshirsagar et al. 1995). Some disadvantages encountered with biological denitrification are rapid changes in the microbial biomass, incomplete denitrification and providing an acceptable, abundant and evenly distributed carbon source (Solt 1986; Dahab 1992). Fluctuation in the carbon or "food" supply or distribution will result in inconsistencies in the amount of nitrate removed.

1.3.3 Phytoremediation

Plants can contribute to soil or water remediation either directly or indirectly. Direct remediation is called phytoextraction, where the plant physically assimilates the contaminant (Kruger et al. 1997). Indirect contributions to remediation refers to plant

enhancement of the role of microbes in contaminant removal. Since plant roots provide a nurturing substrate for microbes, the number and biological function of microbes can increase substantially in plant root zones. It has been demonstrated that degradation of soil contaminants is greater in vegetated compared to non-vegetated soils (Kruger et al. 1997).

When a plant extracts hazardous waste from a substrate, the waste can either accumulate in the plant tissue or it can be processed into another chemical form. The "new" chemical species may be less toxic than the original or rendered completely non-toxic. Transformation of a "harmful" chemical to a benign chemical is ideal. However, undesirable by-products can occur and the plants themselves may need to be removed from the site. Most plants that accumulate a significant amount of metals, often called hyper-accumulators, can be harvested and incinerated. The concentrated metals can then be retrieved and even reused.

In the case of nitrate plants can both directly phytoremediate and enhance bioremediation. Under anaerobic conditions, soil microbes, often associated with plant roots, can transform nitrate to several gas forms including NO, N_2 and N_2O (Mengel 1992). Plants also directly take up and store nitrate in their tissues and transform the nitrate into organic nitrogenous compounds.

One common nitrate phytoremediation technique is riparian buffer zones. These zones are used to intercept and remove nitrate from contaminated water before it enters into a stream or river. Both denitrification and phytoremediation contribute to the nitrate

removal process. According to one study, a red maple riparian buffer zone region removed approximately 120 kg N/ha per year (Nelson et al. 1995)

The biggest advantages to phytoremediation are its low cost and environmental safety. The cost of phytoremediation can be compared to farming since implementation methods are similar. Farming can cost as low as $\$0.05/m^3$ per year while physical and chemical *in situ* remediation ranges from \$10 -\$100 $/m^3$ per year and ex-situ methods can cost between \$30 - $\$1000/m^3$ per year (Cunningham 1997).

Phytoremediation is considered environmentally safe because it does not destroy the soil, it can provide aesthetic value, and since most contaminants can be treated *in situ*, the risk of contamination at other sites as a result of soil transport is reduced (Kruger et al.1997).

Potential disadvantages of phytoremediation are limitations in the contaminant concentration and the depth of the remediation, as well as time needed to complete the project (Cunningham 1997). The depth of the contamination is a concern because if the hazardous waste is beyond the plant's root system, then no phytoextraction can occur. The concentration of the chemical may pose a problem. If the contaminant concentration is too high, the plant might die or internal feedback mechanisms may exclude the contaminant. Finally, time frame limitations may apply. There may be seasonal restrictions or the plant may simply grow too slowly to remove the chemicals within the time constraints of the project. A final consideration in phytoremediation is the need to physically maintain the plants.

CHAPTER 2

NITROGEN AND PLANTS

2.1 The Nitrogen Cycle

The nitrogen cycle is a process where nitrogen is cycled through soil, rock, microbes, plants, animals, and the atmosphere. A loss of nitrogen from one region means a gain of nitrogen in another region. For example, N_2 can be extracted from the atmosphere by bacteria, which ultimately increases soil nitrogen. The nitrogen cycle is complex, but for this thesis the review of the nitrogen cycle will be presented from the perspective of nitrogen availability to the plant.

Nitrate and ammonium become available to plants through weathering of primary soil minerals, mineralization, and nitrogen fixation. Mineralization occurs when organic forms of nitrogen are converted to inorganic ammonia during biodegradation – e.g. the decay of plant materials or animal flesh. The ammonia can either volatilize into the atmosphere, adsorb to soil particles, be absorbed by plants in the form of the ammonium ion or be converted to nitrate by soil bacteria. *Nitrosomonas* bacteria convert ammonia to nitrite (NO_2^-) and *Nitrobacter* complete the process by oxidizing nitrite to nitrate (Lewis 1986).

Nitrogen fixation is the conversion of atmospheric nitrogen (N_2) to ammonia by prokaryotic organisms and is perhaps, the source of most of the world's organic nitrogen (Lewis 1986). Some nitrogen fixing organisms are associated with the roots of plants in a

symbiotic relationship. The microbes provide a form of nitrogen usable to plants while the plants provide carbohydrates for the bacteria (Lewis 1986).

Nitrogen can become unavailable to plants by immobilization or denitrification. Immobilization occurs when soil microbes absorb nitrogen and use it for their own cellular processes. How rapidly this immobilized nitrogen becomes available to plants depends upon the carbon:nitrogen ratio in the soil. If the carbon:nitrogen ratio is high, then there is proportionally less nitrogen available and as a result, the microbes immediately use any mineralized soil nitrogen, leaving little for plants (Lewis 1986).

Immobilization is a temporary sink for plant available nitrogen while denitrification results in a more permanent loss. Denitrification is the process whereby bacteria, under anaerobic conditions, use nitrate as an electron receptor. Nitrate is reduced to dinitrogen gas, nitrous oxide (N_2O), nitric oxide (NO) or nitrogen dioxide (NO_2) (Fowden 1979). Denitrification contributes to a major portion of the loss of inorganic nitrogen (Lewis 1986). When nitrogen is applied to a crop an estimated 15-20% of the nitrogen is lost by leaching or denitrification (Mengel 1992).

When considering a site where nitrate is heavily concentrated in soil and there is the potential for groundwater contamination, the loss of nitrogen from denitrification can be a relatively "positive" event. Unfortunately, some of the by-products of denitrification, such as nitrous and nitric oxide, are greenhouse gases and deteriorate ozone (Mengel 1992).

A significant amount of nitrogen can be also be removed from the soil by plant uptake. In fact, crops utilize approximately 30-80% of the applied nitrogen (Mengel

1992). This is why plants can be used to phytoremediate nitrate contaminated soils. Thus, if a plant extracted just 50% of the nitrate in the soil solution and if 15% of the nitrate were denitrified, then 65% of the nitrate could potentially be removed.

2.2 Plant Physiology and Nitrate

Nitrogen is an essential plant nutrient and contributes greatly to plant growth (Hageman 1979). Nitrogen is a key component in the fundamental building blocks of plant matter and is used to build amino acids, proteins, nucleic acids, alkaloids and phytohormones (Hageman 1979; Lewis 1986). In fact, approximately 0.5-5% of the total dry plant matter is nitrogen (Tinker 1979; Larsson et al. 1992).

Hageman (1979) notes that an "adequate N supply is considered to be essential in the establishment of the yield capacity" of a crop. Dale (1979) found that a delay in nitrogen application reduced the leaf size in cereal seedlings by 25% and total soluble protein was reduced by approximately 35%. The general trend is the same for most plants – increased available soil nitrogen results in increased plant growth.

Increased plant growth is an important characteristic to consider in phytoremediation, especially in the case of nitrate removal. The logic is that as the plant grows larger, the more nitrogen is used and the more nitrate is removed from the contaminated soil. Along a similar line, one can consider harvesting the plant to move the nitrogen off premises and encourage new growth, which will encourage continued nitrate absorption.

2.2.1 Differences in Plant Response to Nitrate

Research on crop response to nitrate has demonstrated that genetic variations between species and varieties within species can influence the crop yield, the location of nitrate and nitrogen accumulation within the plant, the extent of nitrate and nitrogen accumulation in plant tissues, plant uses for nitrate and nitrogen, and the rate at which nitrate is reduced within the plant (Campbell 1979).

Wheat removes approximately 50% of the applied nitrogen while other grains remove about 30% (Mengel 1992). Assuming these crops were grown under the same environmental conditions and initial nitrogen losses from leaching and denitrification were equal, this data demonstrates that there are genetic differences between plants in their need for nitrogen and/or their ability to extract or store nitrogen.

When several genotypes of pearl millet and wheat were exposed to different nitrate concentrations, Gupta et al. (1978) found that the yield of all the varieties increased as nitrogen application increased, but there was variability among the hybrids. Stulen et al. (1990) and Stewart et al. (1987) also showed that nitrate accumulation in roots, leaves and petioles varied between varieties and species.

When managing a cash crop, the goal is to use plant varieties which utilize nitrogen efficiently in order to reduce fertilizer applications, thereby decreasing costs and groundwater contamination. Conversely, when using plants to phytoremediate a nitrate contaminated region, it is desirable to have plants that can rapidly assimilate and accumulate large amounts of nitrogen without adverse affects to the plant. In fact, several

factors need to be considered when assessing a plant species' potential to remediate a nitrate contaminated environment, including survivability and the plant's ability to assimilate and accumulate nitrate.

2.2.2 Plant Survivability

When selecting a plant species to use in phytoremediation, one must first determine whether the plant species will survive in the given environment. This not only includes considering the region's temperature, rainfall, relative humidity, soil type, and pH, but also whether the plant can tolerate the chemical it is intended to remediate; in this case very high concentrations of nitrate. Working with plant species which are native to the region is an appropriate way to address the climatic factors. This is one reason why fourwing saltbush and greasewood are being considered to revegetate and remediate the Monument Valley UMTRA site.

Though the climatic factors are not an issue with these plants, we were concerned that plants adapted to desert environments, where soils typically have low concentrations of nitrate, may not be able to survive excessive amounts of nitrate. However, fourwing saltbush (*Atriplex canescens*)has been shown to be a particularly hardy plant that can rapidly adapt to extreme conditions such as high temperature, high soil salinity, and low moisture (Doria and Aldon 1993). Also, some *Atriplex* species have been identified as

"nitrophylic" and accumulate high tissue concentrations of nitrate (Osmond et al. 1980). Unfortunately, less is known about greasewood and its ability to assimilate nitrate.

2.2.3 Nitrate Assimilation by Plants

How rapidly plants assimilate nitrate is important to consider for phytoremediation of nitrate contaminated soil. Plants that can rapidly absorb nitrate are an advantage because nitrate loss from leaching is reduced.

Plants absorb nitrogen primarily through their roots and then incorporate it into proteins, nucleotides and other nitrogen-based compounds (Goodman 1979). A plant's nitrate assimilation rate is influenced by both external and internal factors. External influences include soil moisture content (Sinha and Rajgopal 1978; Varma 1978) and the concentration of nitrate in the soil solution (Eilrich and Hageman 1973). Internal controls of nitrate assimilation are primarily associated with the nitrate reductase enzyme (NR) (Abrol and Nair 1978), storage mechanisms within the plant, and plant growth rate.

It makes sense that the soil moisture content could affect a plant's nitrate assimilation rate since most plants absorb ions from the soil solution (Osmond et. al 1980). Varma (1978) found that, in aerobic conditions, nitrogen uptake increased with soil moisture. A positive correlation also exists between external nitrate concentration and nitrate uptake.

The presence and concentration of nitrate in the soil solution is closely connected with the internal controls, including stimulation of the NR enzyme and storage of nitrate. In 1973, Eilrich and Hageman found that the nitrogen content in grains was positively correlated with both NR activity and the amount of nitrogen applied to the plant.

Since nitrate is not directly utilized by plants, it is stored and eventually converted to ammonium by a two step method. NR enzymes reduce nitrate to nitrite and then nitrite is converted to ammonium by nitrite reductase enzymes (NiR) (Maldanado, et al. 1990). It is generally agreed that NR activity is stimulated by the presence of nitrate in the nutrient solution (Hucklesby, et al. 1990; Maldanado et al. 1990) and that NR at first appears slowly and then more rapidly (Campbell 1987). This phenomenon is generally referred to as nitrate induction of NR activity. Though NR activity appears when nitrate is present, the concentration of nitrate in the soil solution has "a small or negligible effect on the induction of NR" (Wallace 1987). However, up to a point, the steady-state activity of the NR enzyme increases as the external nitrate concentration increases (Wallace 1987).

Research conducted by Larsson et al (1992) indicate that plants have a nitrate feedback mechanism. They found that nitrate influx rates in several plant species "were proportional to the resulting tissue nitrate concentration up to" a certain amount, then the influx decreased. The feedback mechanism is probably closely related to the plant's storage capacity.

2.2.4 Nitrate Accumulation and Storage in Plant Tissue

A plant's ability to store nitrate for future use is an important survival mechanism since nitrate availability is often limited. This feature may also be important in phytoremediation of nitrate contaminated soils. For example, some plants may have extensive nitrate storage capability, which could increase the quantity of nitrate removed from the soil. Many *Atriplex* species are called "nitrophilous" plants and have been shown to contain very high leaf nitrogen contents (Osmond et. al 1980), which could make them excellent candidates for phytoremediation. The ability of *Atriplex* spp. plants to accumulate and store large quantities of nitrate is probably due to the need to take advantage of short periods of nitrate availability during brief wet periods (Osmond et. al 1980). I hypothesize that greasewood will have similar abilities.

Whether a plant immediately reduces nitrate or stores it depends on several factors including the location of nitrate within the plant, the plant's age, and nitrate concentration of the plant tissue, the rate of nitrate uptake versus the rate of reduction and transport, light availability and temperature (Tinker 1979; Larsson et al. 1992; Goodman 1979).

The region of the plant where nitrate is stored appears to be species dependent. Typically, woody plants reduce nitrate in the root, but in many C-4 plants reduction occurs in the shoots. In "sun species" nitrate reduction often occurs in the leaf (Stewart et al. 1987).

Within the cell, the storage of nitrate in vacuoles seems to occur under high nitrate, low light and high temperature conditions or when nitrate uptake or transport exceeds reduction capacity (Goodman 1979; Clement et al. 1979). Nitrate exists in two "pools" in a cell, an active or metabolic pool, and a storage pool. Aslam et. al (1976) found a "linear correlation between nitrate reductase activity and the size of the metabolic nitrate pool," supporting the theory that it is the nitrate in the metabolic pool, not the storage pool, that is responsible for NR induction. If maximum reduction rates are to be maintained, then the nitrate in the metabolic pool needs to be continuously replenished by root uptake (Clement et al. 1979). Otherwise, the plant has to essentially switch functions to access the nitrate in the storage pool.

Tinker (1979) showed that the total crop nitrogen often exceeded the amount of applied nitrogen. This is because the plant absorbs nitrogen over its lifetime, except during reproduction and death, and once the applied nitrogen is gone the plant absorbs additional nitrogen from remineralization of nitrogen already in the soil or nitrogen fixation. Tinker lists several examples of total crop nitrogen compared with applied nitrogen. In one study, sugar beet had a total crop nitrogen of about 200 kg/ha while the applied N was 150 kg/ha. Winter wheat accumulated 140 kg/ha of nitrogen while only 93 kg/ha was applied.

2.2.5 Calcium and Ammonium as Potential Nitrate Uptake Inhibitors

Research suggests that nitrate and calcium interact to affect relative concentrations of the other, especially in the leaf. Nitrate has been found to increase the calcium concentration in plant tissue (DeKock et al. 1979). However, when several crop plants were exposed to higher external calcium concentrations, the calcium concentration in the leaves increased while the nitrate concentration decreased (DeKock et al. 1979). DeKock et al. also found that the total plant nitrogen content varied with nitrate supply but "was only slightly affected by calcium." This effect may be significant at the Monument Valley UMTRA site because calcium occurs in extremely high concentrations in the soil .

Another factor that may inhibit nitrate assimilation is the presence of ammonium. In general, nitrate utilization and uptake decrease when both nitrate and ammonium are in the soil solution (Maldanado, et al. 1990; Pilbeam and Kirkby 1992). In fact, at a basic pH, most plants will preferentially remove ammonium over nitrate (Pilbeam and Kirkby 1992). This is probably because it takes less energy for a plant to uptake and process ammonium compared with nitrate (Lewis 1986).

When most plants are grown with ammonium as their only nitrogen source, the plants are much smaller (Lewis et al. 1987). There is debate about the exact effect nitrate and ammonium have on plant growth because growth response appears to be species dependent. Even though many plants grow better when supplied nitrate (Pilbeam and Kirkby 1992; Fowden, L. 1979), there is evidence that the growth rate can be increased when both nitrate and ammonium are supplied simultaneously (Pilbeam and Kirkby

1992). Carob plants, however, have been shown to grow best in ammonium only or nitrate+ammonium solutions (Martins-Loucao and Duarte 1987). The ammonium-only response carob displays seems to be the exception for plants rather than the rule.

The combined effect of nitrate and ammonium on fourwing saltbush and greasewood is unknown and is a potential concern at the Monument Valley Site. If ammonium decreases plant nitrate uptake, then the rate of nitrate remediation may decrease and the nitrate will be transported to the groundwater. On the other hand, if the ammonium is continually being converted to nitrate, rather than volatilized, then the preferential extraction of ammonium will be beneficial by preventing the development of more nitrate. Also, if fourwing saltbush and greasewood show increased growth when both nitrate and ammonium are present, compared with nitrate alone, then a greater amount of nitrogen can be removed from the system.

CHAPTER 3

THE EXPERIMENTAL DESIGN

A two-month greenhouse-based experiment was conducted from June 1998 to the end of July 1998 to determine if fourwing saltbush and greasewood could tolerate high nitrate concentrations (up to 2000 mg/L) and to quantify each plant's potential to remove nitrate. The plants were exposed to five different nitrate concentrations (tap water, 50 mg/L, 100 mg/L, 750 mg/L and 2000 mg/L as nitrate) in two different soils, one from Monument Valley, AZ the control soil from Tuba City, AZ. Several methods were used to measure the plant response to nitrate, including wet and dry mass of the roots, stems and leaves, transpiration, leaf nitrate concentration, percent total nitrogen (excluding roots), initial and final soil nitrate, and leachate nitrate concentration. The leachate nitrate concentration was measured at the beginning, middle, and end of the experiment.

Comparably sized three-month old fourwing saltbush and greasewood were planted in 7.6 liter drainage lysimeters each containing 11 kg of air-dried soil. The drainage lysimeters were labeled and distributed in a completely random design. A starting wet and dry mass of four representative samples each of fourwing saltbush and greasewood were obtained. The plants were oven dried at 80° C to a constant mass to obtain dry mass.

Five levels of nitrate (Tucson tap water, 50 mg/L, 100 mg/L, 750 mg/L and 2000 mg/L as nitrate) were each applied to 40 fourwing saltbush and 40 greasewood plants (4 repetitions). Each plant was irrigated with two liters of the nitrate solution every other

day in order to avoid over watering the plants and still maintain consistent nitrate concentrations in the moist soil. A quarter strength modified macronutrient Hoagland solution containing KH_2PO_4, KCl, $MgSO_4$, and chelated iron was included in the treatments to minimize the possibility that plant response was a function of inadequate nutrients. All nitrate and ammonia-based compounds were excluded from the Hoagland solution. The micronutrients B, Mn, Zn, and Cu were applied at full strength. Fifty mg/L of NaCl was also added to all treatments to approximate the mean salt concentration found in the Monument Valley site groundwater supply.

After each plant was irrigated, the soil was allowed to drain for 48 hours and the volume of leachate was measured to obtain transpiration rates. Evaporation was minimized by surrounding the pots and the base of each plant with plastic film covered with fitted styrofoam sheets. Baseline water loss was monitored by applying tap water to 8 drainage lysimeters containing only soil. The soil-only lysimeters were also covered with plastic and styrofoam to minimize evaporation. The pan evaporation and greenhouse temperature were monitored regularly (high temperature of 40° C and low temperature 27°C) plant height, width, stem width and observational data, such as color, were recorded weekly, and leachate samples were collected for nitrate analysis at the beginning, middle and end of the experiment.

Upon conclusion of the experiment, wet and dry weights of the leaves, stems and roots of each plant were measured. Five leaves per plant were removed to determine the leaf area index.

Chemical analysis was conducted by Laboratory Consultants, Ltd. of Phoenix,

Arizona and includes measurement of nitrate in the soil, leachate, and leaves and total nitrogen excluding the roots. The water samples were preserved with 0.5 mL HCl. The lab was requested to treat the samples with Ag_2SO_4 to remove any chloride interference prior to measurement. Nitrate was measured with an Ion Selective Electrode and the total percent nitrogen was measured in the leaves and stems using the Kjeldahl method. Standard method digestions were performed.

The data was analyzed using Statview 5.0 for Macintosh as a 2-way and 3-way Analysis of Variance (ANOVA). In the 2-way ANOVA the factors included plant type and soil, while nitrate concentration was included as a co-variant. In the 3-way ANOVA nitrate concentration was included as a factor with plant type and soil. For all tests the null hypothesis was rejected when $p \leq 0.05$ (95% confidence level).

CHAPTER 4

EXPERIMENTAL RESULTS

Both fourwing saltbush and greasewood survived nitrate concentration levels as high as 2000 mg/l, though greasewood showed reduced growth at the highest nitrate level. Also, both species absorbed similar quantities of nitrogen, but managed nitrogen differently.

The soils were 84-88% sand, 5-6% silt, and 7-10% clay and were initially analyzed for ammonia (NH_3), nitrate (NO_3), and cations. Table 4.1 provides a summary of the initial soil analysis. The Monument Valley soil contains approximately twice the amounts of nitrate, ammonia, available nitrogen, and exchangeable calcium as the Tuba City soil.

Table 4.1, Initial Soil Analysis Results

Lab Tests	Monument Valley	Tuba City
pH	7.2 ± 0	7.8 ± 0
EC, ds/m	3.7 ± 1.1	0.5 ± .1
Free Lime	Yes	No
Organic Matter, %	0.6 ± 0.1	0.4 ± 0
Nitrate, mg/kg	172 ± 32	48 ± 8
Available N, kg/A mg/kg	80 ± 5	29 ± 3
Available P, mg/kg	16 ± 4	3 ± 0
Exchangeable K, mg/kg	64 ± 5	93 ± 2
Exchangeable Mg, mg/kg	182 ± 10	121 ± 1
Exchangeable Ca, mg/kg	4931 ± 9	2048 ± 49
Exchangeable Na, mg/kg	27 ± 1	22 ± 1
Exchangeable Na Percentage, %	0.4 ± 0	0.8 ± 0
NH_4, mg/kg	6.1 ± 3.4	3.4 ± 0.4

A total of 4 out of 80 plants died during the eight week experiment 3 greasewood and 1 fourwing saltbush. All of the plants that died were in the Tuba City soil. The greasewood died in the 5th, 6th, and 7th weeks of the experiment on the two lowest nitrate levels. The first and third greasewood plants that died received the tap water-only treatment and the plant that died in the 6th week received the 50 mg/L nitrate treatment. The fourwing saltbush died from a root fungus two days before the end of the experiment. It received 750 mg/L of nitrate (Level 4) and was one of the largest plants. The data obtained on each plant was included in the averages up to the point of death.

4.1 Plant Mass

Table 4.1.1 shows the average dry mass of both species for each nitrate application level in each soil. No significant difference was found between plant mass and soil type (Monument Valley and Tuba City). A significant association was found between plant mass and nitrate concentration.

The initial total dry mass of representative samples of greasewood, including roots, stems and leaves, was 1.1 g. Thus, with the exception of the plants that died, all plants grew a significant amount ($p=0.0019$).

Greasewood's dry masses at Level 4 are marginally larger than all the other levels (Fig. 4.1.1), which suggests its nitrate optimum is 750 mg/l. On the other hand, fourwing saltbush's largest growth was at 2000 mg/L nitrate.Based on the dry masses of both species, fourwing saltbush grew significantly more than greasewood (dry mass $p=0.0002$).

TABLE 4.1.1, Total Dry Masses of Plants Grown on 2 Soil Type at 5 Different Nitrate Levels

	Fourwing Saltbush		Greasewood	
	Dry (g)		Dry (g)	
STARTING MASS	1.5 ± 0.2		1.1 ± 0.2	
SOIL TYPE	MV	TC	MV	TC
LEVEL 1 (tap water)	5.27 ± 2.07	3.99 ± 1.03	3.05 ± 0.28	2.53 ± 0.62
LEVEL 2 (50 mg/L NO_3^-)	6.99 ± 2.45	7.58 ± 1.55	3.54 ± 1.14	2.72 ± 1.03
LEVEL 3 (100 mg/ L NO_3^-)	9.12 ± 2.83	5.00 ± 1.04	4.65 ± 2.72	3.11 ± 1.63
LEVEL 4 (750 mg/ L NO_3^-)	8.79 ± 4.00	9.24 ± 5.2	6.89 ± 3.73	7.27 ± 4.67
LEVEL 5 (2000 mg/ L NO_3^-)	14.48 ± 2.03	10.80 ± 3.57	4.12 ± 0.88	3.74 ± 1.67

	PLSD for Total Dry Mass P-Value
Soil Type	0.1631
Plant Type	0.0002

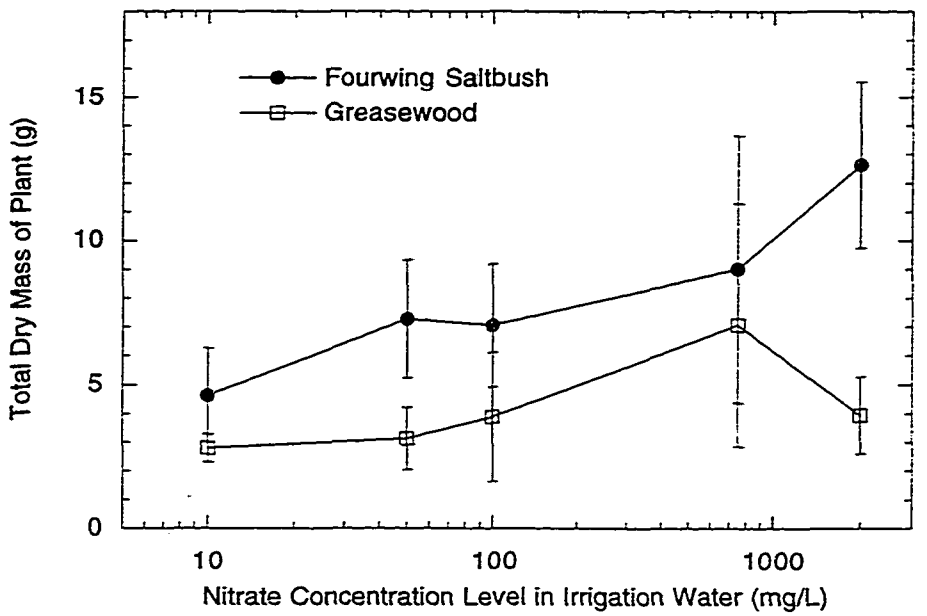

FIGURE 4.1.1, Combined Average Dry Masses of Plants Grown with 5 Different Nitrate Levels

4.2 Transpiration

The transpiration (water loss) of the two plant species was measured in order to form a Nitrogen Removal Efficiency Index, which compares the nitrogen utilized per volume of water used by each plant. The transpiration data was obtained by taking the difference between the volume of water applied and the volume of water that leached through the soil, minus the water loss from the soil only pots:

$$T = (L_i - L_f) - (L_i - L_c)$$

where L_i is the total amount of water applied during the experiment after initial soil saturation, L_f is the total amount of water that drained from the pot, and L_c is the average water lost from the soil-only pots. No significant differences were found in plant transpiration over nitrate levels or plant type (Figure 4.2.1). It is noteworthy that despite the higher growth of fourwing saltbush it had a lower transpiration than greasewood at all nitrate levels.

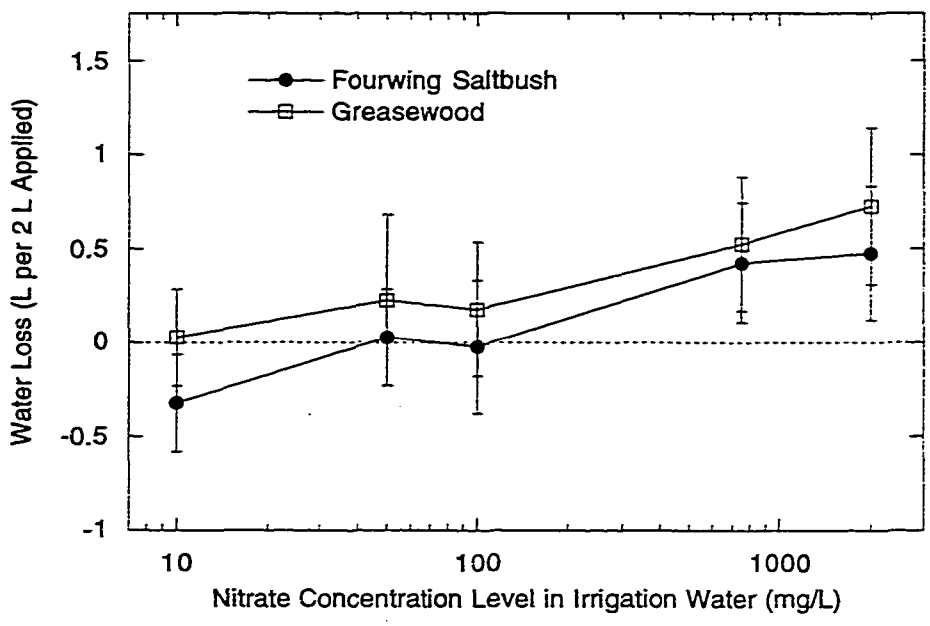

FIGURE 4.2.1, Transpiration (water loss in L per 2 L applied) versus the nitrate concentration in irrigation water.

Water levels were measured after each irrigation for the entire length of the experiment. The data was combined into weekly increments and examined for differences transpiration over time and then completely combined to determine the total average transpiration. Thus, the total average transpiration reflects an average of younger and older plants, which may account for some of the variability in the data. Water that was spilled during application or leaked from cracked pots is another potential source of error. This would mostly result in an over estimate of transpiration except when the leaks or spills occurred in the soil-only pots, in which case the error would make it appear as though the transpiration were lower.

4.3 Plant Nitrogen

The concentrations of leaf nitrate and total nitrogen for both plant species showed a significant increase with the level of nitrate applied (p=0.0002) (Table 4.3.1). Thus, nitrate and nitrogen accumulation were greatest at the two highest nitrate levels, 750 mg/L and 2000 mg/L and greasewood had a significantly higher total nitrogen percentage than fourwing saltbush (p=0.0003) The leaf nitrate levels in greasewood were also greater than in fourwing saltbush, but fell just below the 95% significance level (p=0.06). Figure 4.3.2 shows tissue nitrate versus concentration. The increase in tissue nitrate between Level 4 and 5 is significant (0.0003). There was no significant difference between soil types (p=0.2238).

TABLE 4.3.1, Tissue Nitrate and Total % N levels

	Fourwing Saltbush				Greasewood			
	Nitrate (ppm)		Total % N		Nitrate (ppm)		Total %N	
	MV	TC	MV	TC	MV	TC	MV	TC
LEVEL 1 (tap water)	208 ±55	162 ±6	1.4 ±1.1	1.6 ±0.8	316 ±135	221 ±40	2.1 ±0.4	3.7 ±2.2
LEVEL 2 (50 mg/L NO_3^-)	219 ±72	189 ±44	1.7 ±0.4	1.2 ±0.2	235 ±63	271 ±122	2.4 ±0.4	2.0 ±0.5
LEVEL 3 (100 mg/ L NO_3^-)	189 ±26	245 ±154	1.7 ±0.2	2.5 ±1.3	217 ±35	295 ±86	2.9 ±0.9	2.9 ±1.2
LEVEL 4 (750 mg/ L NO_3^-)	477 ±225	344 ±133	2.1 ±0.7	2.7 ±0.4	783 ±344	361 ±111	4.1 ±0.3	3.8 ±0.9
LEVEL 5 (2000 mg/ L NO_3^-)	547 ±368	608 ±244	2.8 ±0.1	3.1 ±0.2	1255 ±301	788 ±656	4.2 ±0.3	3.6 ±0.8

	PLSD for Tissue Nitrate P-Value
Soil Type	0.2238
Plant Type	0.0604

	PLSD for Plant Nitrogen P-Value
Soil Type	0.4971
Plant Type	0.0003

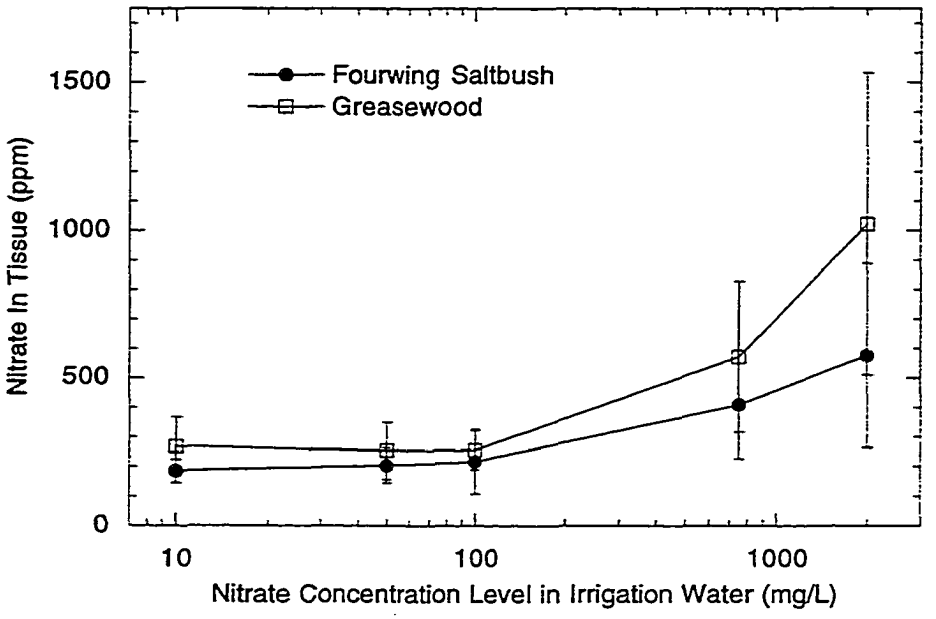

FIGURE 4.3.2, Tissue nitrate vs. nitrate concentration level in the irrigation water

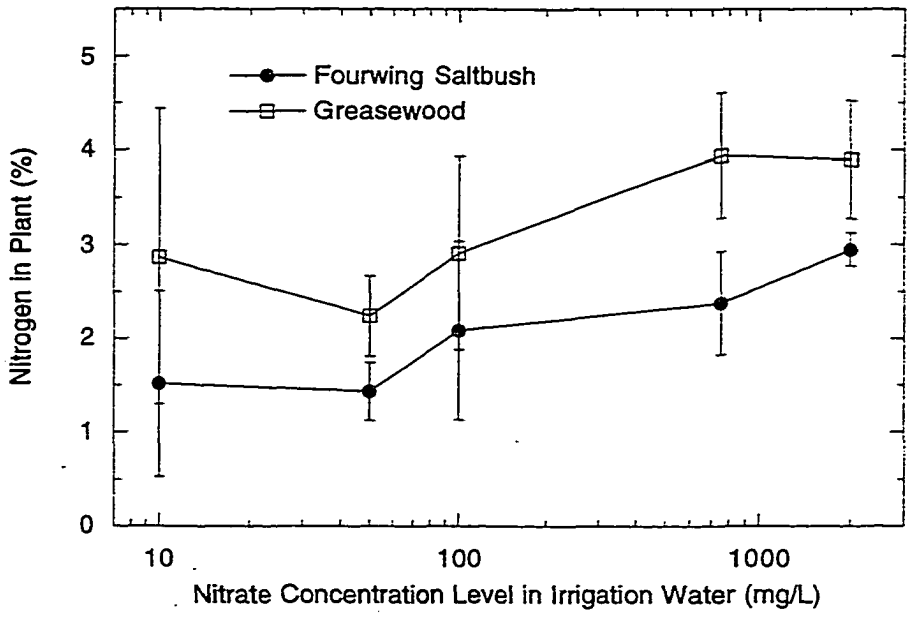

FIGURE 4.3.3, Tissue nitrogen versus the nitrate concentration in irrigation water

4.4 Soil and Leachate Nitrate

The initial nitrate concentration for the Tuba City soil averaged 48 ± 4 mg/kg. The Monument Valley soil was more variable and ranged from 172-229 mg/kg because the soil may not have been mixed well . The average final soil nitrate ranged between 7 and 114 mg/kg and increased with the applied nitrate level for both soils.

Leachate nitrate levels were measured at the beginning, middle and end of the experiment, to determine if there was an increase in nitrate extraction over time. The "beginning" data set were excluded from the analysis because the leachate nitrate concentrations from the Monument Valley soil showed considerable variability in nitrate level (Figure 4.4.1). Though a small trend emerged between the middle and final analyses, no significant difference between nitrate concentrations were found (Figure 4.4.2).

The variability in the initial nitrate leachate concentrations suggests that there was a greater range of nitrate in the Monument Valley soil than the original soil analysis revealed.

The nitrate that was initially in the Monument Valley soil was eventually leached out and, at least by the second collection, the leachate nitrate levels reflected the concentrations introduced through treatments. It is assumed that the nitrate contributed by the soil was leached quite rapidly, since both soils are 84-88% sand and nitrate travels rapidly through most soils. It is also assumed that the initial presence of nitrate did not

significantly affect the growth or nitrate tissue levels of the plants since no significant growth differences were found between the two soil types.

4.5 Nitrogen Removal

The quantity of nitrogen accumulated in the plant was accepted as a measure of nitrogen removed from the system. The nitrogen removal potential can be calculated by multiplying the percent nitrogen by an estimated biomass production over time. The minimum and maximum average percent nitrogen in fourwing saltbush is 1.2% and 3.1% and in greasewood it is 2.1% and 4.2%. The percent nitrogen range accounts for the differences in nitrogen accumulation as a function of concentration.

Assuming that once the plants are mature they can produce 10,000 kg of dry shoot biomass per hectare per year, a number based on measurements of biomass production for *Atriplex nummularia* (Glenn et al. 1998), then fourwing saltbush would use between 120 and 310 kg N per hectare per year and greasewood would use between 210 and 420 kg N per hectare per year. Comparatively, Nelson et al. (1995) estimated that a riparian nitrate-buffer zone removed approximately 120 kg N/ha/yr.

Realistically, the kg of N removed by greasewood is probably closer to the estimates for fourwing saltbush because of its slower growth. In fact, my results indicate that fourwing saltbush and greasewood do not differ in their total mass of nitrogen per gram of plant (Figure 4.5.1). The total nitrogen mass was calculated by multiplying the percent tissue nitrogen and the dry mass.

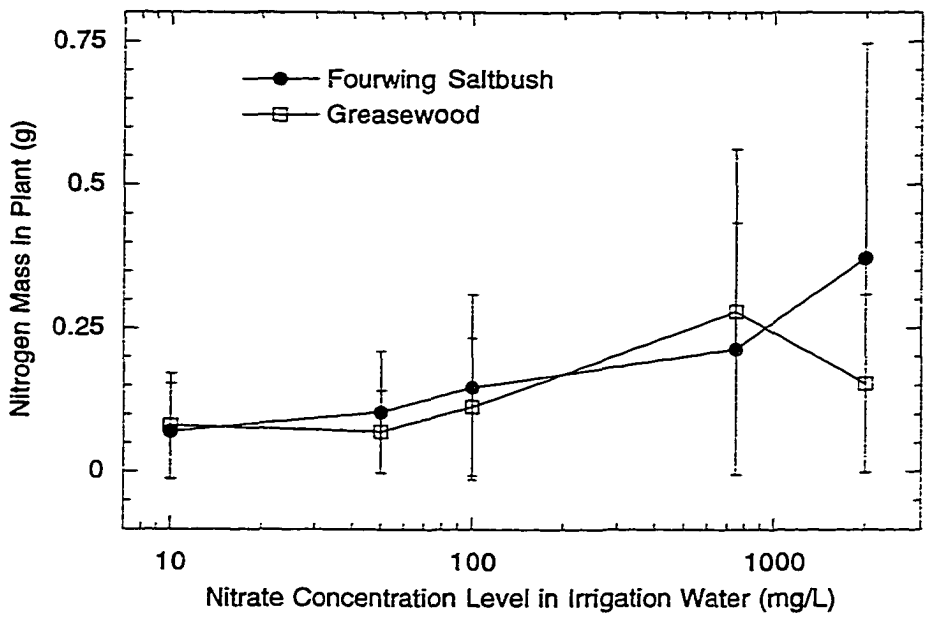

FIGURE 4.5.1, Nitrogen Mass in Plant per Nitrate Concentration Level

Figure 4.5.1 also shows a significant association between the plant nitrogen mass and the concentration of nitrate.

If water remediation is a goal, then transpiration becomes an important variable when estimating the remediation potential of the plant. If the plant has low transpiration and can extract large quantities of nitrate, then proportionally more nitrate may be removed compared with the water and remediation may occur. In principle, a Nitrogen Removal Efficiency Index for a plant can be constructed from the total mass of nitrogen extracted by the plant and the transpiration, and reflects the plant's efficiency in nitrate extraction and utilization. The relation is given by

$$(\%N)(M_{ag})/T = \text{mg N utilized/L water utilized},$$

where %N is the percent nitrogen in the upper portions of the plant, M_{ag} is the final above ground dry weight of the plant, and T is the transpiration (water use). Unfortunately, in the current data, the large uncertainties in T render this index insensitive to variations between the plants.

CHAPTER 5

DISCUSSION

The differences between both the wet and dry masses and nitrate and percent nitrogen tissue concentrations of greasewood and fourwing saltbush demonstrate a species specific growth response and tolerance to nitrate, as well as a difference in their ability to convert nitrate to organic forms. Overall, however, there was no significant difference between the total mass of nitrogen processed by each plant species. Fourwing saltbush showed a positive growth and tissue accumulation response to nitrate concentration, with its largest mass and tissue concentrations correlating with the highest nitrate level (2000 mg/L as nitrate). Thus, fourwing saltbush demonstrated a remarkable ability to tolerate and process large quantities of nitrate at very high concentrations. Greasewood, on the other hand, had its growth optimum at a lower nitrate concentration (750 mg/L as nitrate).

Based on dry mass, both plant species showed lower growth at the lowest nitrate levels. This is consistent with the research by Hageman (1979) which states that "adequate N supply is considered to be essential in the establishment of the yield capacity" (see Section 2.2).

Although the wet and dry masses of greasewood did not continually increase with external nitrate concentration, the nitrate tissue concentration did increase but total nitrogen tissue concentration increased only up to 750 mg/L (Table 4.3.1). It appears that greasewood has an upper limit for processing nitrate. It also appears that greasewood

does not have a feedback mechanism to prevent continued nitrate accumulation even though it reached potentially growth-inhibiting levels.

These effects may be explained by some of the research cited in Chapter 2 of this thesis. For example, Wallace (1987) found upper limits to the steady-state activity of the nitrate reductase enzyme as a function of the external nitrate concentration, i.e. NR activity increased as the external nitrate concentration increased up to a point. Thus, based on my results, greasewood's maximum NR steady-state activity may be somewhere around 750 mg/L external nitrate concentrations. Whether this is actually the case needs to be determined by conducting a physiological study where the NR activity is measured and there are smaller increments of external nitrate concentrations between 750 mg/L and 2000 mg/L.

Fourwing saltbush differs from greasewood in that its total percent nitrogen is comparatively lower, but the total percent nitrogen continues to increase with concentration. This might suggest that fourwing saltbush can rapidly process nitrate even at very high external concentrations and apply the products towards growth. Rapid growth may be an important nitrate management mechanism for fourwing saltbush under such extreme concentrations.

Whatever mechanisms a plant uses to uptake, store, and process nitrate, they all contribute to the ultimate ability of the plant to remove nitrate from the soil solution. An important relationship to consider is a combination of the plant growth, transpiration, and its ability to remove and process nitrate into organic nitrogen. These factors can be represented through a Nitrogen Removal Efficiency Index (as defined in Section 4.5).

Plants with a high Nitrogen Removal Efficiency Index will be able to remove most of the nitrate without utilizing all of the water. Though my data could not differentiate between the transpiration of greasewood and fourwing saltbush, other research, such as Nichols (1994), has shown that greasewood has a significantly higher transpiration that fourwing saltbush. If so, then fourwing saltbush would have a comparatively better Nitrogen Removal Efficiency Index and may be able to better minimize nitrate accumulation in the soil from irrigation with the nitrate-rich groundwater.

In the field, evaporation also needs to be considered. If evapotranspiration, the combination of evaporation and transpiration, exceeds the rate at which nitrate is extracted by the plant, then nitrogen removal may be hindered because much of the nitrate will be left behind in the soil before the plant has the opportunity to extract it from the water.

The evapotranspiration at the Monument Valley site may be higher than that of the greenhouse, so an Adjusted Nitrogen Removal Efficiency Index that considers evaporation may need to be developed specifically for this site.

Determining a plant's ability to tolerate and remove high concentrations of nitrate is a first step in estimating the potential of using nitrate contaminated groundwater to aid revegetation efforts. The loss of nitrate from denitrification, as well as the ultimate fate of the nitrogen that has been extracted by the plants, such as removal though animal grazing or reintroduction from litter fall or urea, also need to be considered. Harvesting the plants is a simple way to remove the nitrogen from the site and to encourage new

plant growth, which may enhance the nitrate removal process. Harvesting could stop once the site reaches acceptable nitrate levels.

CHAPTER 6

CONCLUSIONS AND FUTURE RESEARCH

Fourwing saltbush and greasewood have demonstrated species-specific responses to exposure to five concentrations of nitrate. Greasewood's growth was optimal at nitrate 750 mg/L, but declined at 2000 mg/L. Fourwing saltbush, on the other hand, showed remarkable tolerance up to 2000 mg/L.

Though there is no significant difference in the mass of nitrogen removed by fourwing saltbush and greasewood at a given concentration level, there are apparent differences in their management of nitrate. Fourwing saltbush was able to process the nitrate at all levels and apply the nitrogen toward growth. Greasewood, on the other hand, accumulated larger amounts of tissue nitrogen and nitrate. Overall, greasewood seems to be limited by slower growth, environmental sensitivity, and appeared to have a smaller range of nitrate tolerance compared with fourwing saltbush.

These differences are important when considering which plant to use to revegetate and phytoremediate the site. Fourwing saltbush should be the priority plant because of its ability to remove nitrate, grow quickly, and tolerate extreme conditions, be it salt, draught, or excessive nitrate levels. The ability to tolerate extremely high nitrate levels is particularly important at the Monument Valley site because, if the plants are irrigated with the contaminated groundwater, they may be exposed to more than 2000 mg/L of nitrate. Thus, it would be useful to expose fourwing saltbush and greasewood to nitrate

concentrations greater than 2000 mg/L to determine each species upper tolerance limits for nitrate.

Though my investigation provides a starting point for the development of a revegetation and a phytoremediation plan using greasewood and fourwing saltbush, there are still several issues that need to be addressed in order to complete the phytoremediation program at the Monument Valley UMTRA site. Most important are to assess ammonia toxicity, and the affect of ammonia on nitrate removal. In addition, it would be useful to determine a total biomass production for greasewood and fourwing saltbush, as well as, a total loss of nitrate, which includes denitrification.

Other important future research includes developing an irrigation regime that promotes denitrification and optimizes nitrate uptake by the plants. The regime would also need to consider the remediation goals at the site - i.e. soil and water remediation or just soil remediation. If the primary goal is soil remediation, then the irrigation plan would need to consider the amount of application to minimize leaching past the root zone.

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IMAGE EVALUATION
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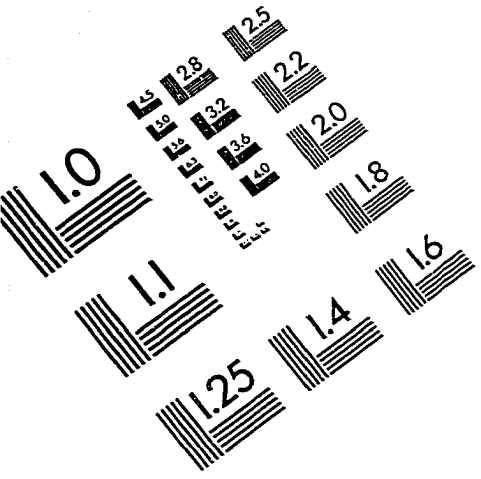

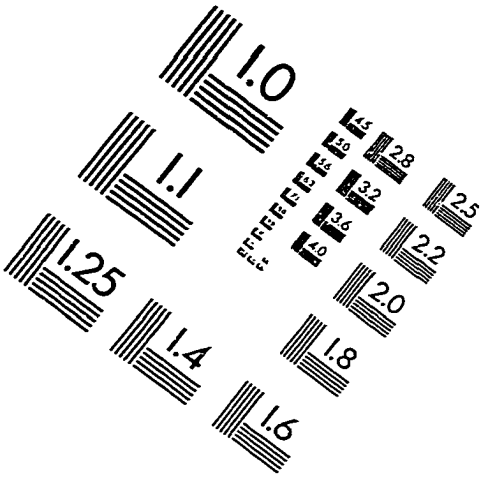

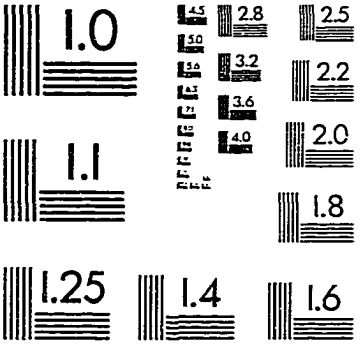

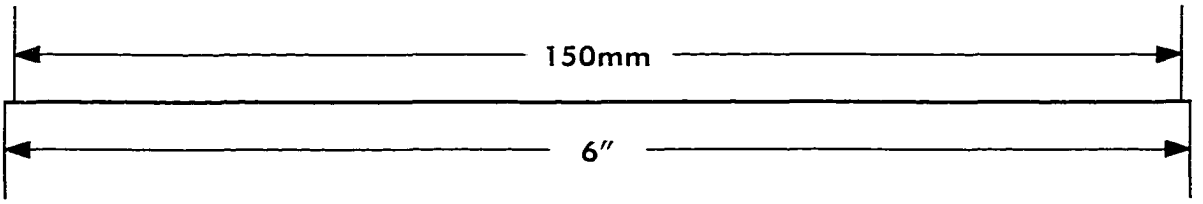

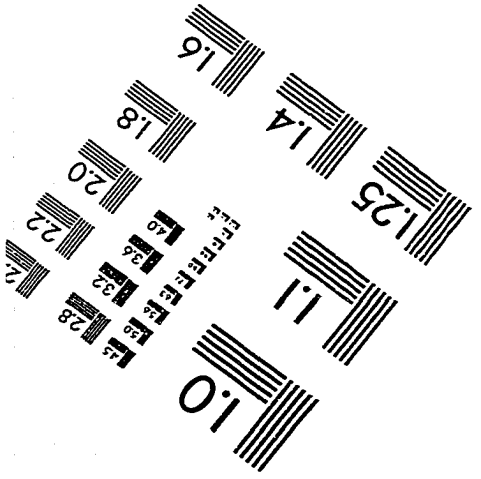

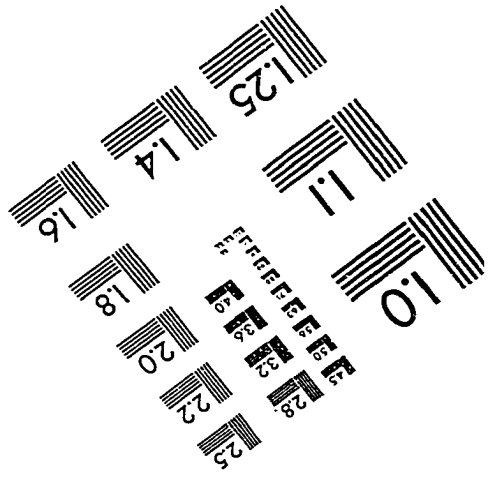

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