INFLUENCE OF AMMONIACAL-N ON WETLAND PLANT SPECIES[1]

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Ammoniacal-nitrogen (N) is a common constituent of mine waste residues, industrial wastewater, and agricultural runoff. The purpose of this study was to evaluate the phytotoxic potential of ammoniacal-N contaminated groundwater encountered at a Department of Energy (DOE) wetland mitigation site near Rifle, Colorado. A series of experiments were conducted using site water modified to represent ammoniacal-N levels ranging from pure site water to dilutions containing 50%, 25%, 10%, and 0% site water. In addition, pH was modified to represent potential site conditions. Studies conducted included: 1) a germination study to evaluate seed response to elevated ammoniacal-0N levels; 2) a growth chamber study to evaluate initial seedling growth in response to elevated ammoniacal-N levels; and 3) a greenhouse study to evaluate mature seedlings (>16 weeks old) growth in response to elevated ammoniacal-N levels. Seed germination and initial seedling growth were affected by levels of 100 ppm ammonium-N or less with resulting reductions in germination percentage, reductions in root and shoot growth, and browning of roots. Mature seedlings were affected by high ammoniacal-N levels in three of five wetland species as evidenced by low shoot biomass levels, chlorotic leaves, leaf loss, and drought-like symptoms. *Typha latifolia* and *Scirpus acutus* were tolerant of levels up to 400 ppm ammonium. *Populus fremontii* and *Carex nebraskensis* demonstrated tolerance of ground water levels up to 200 ppm ammonium. *Salix exigua* was impacted by ammonium levels as low as 100 ppm. Overall, this study demonstrated the need for the consideration of the potential phytotoxic effects of ammoniacal-N as part of restoration planning efforts.

Additional Key Words: ammonium, ammonia, phytotoxicity, *Salix exigua*, *Typha latifolia*, *Scirpus acutus*, *Carex nebraskensis*, *Populus fremontii*, *Puccinellia airoides*, *Deschampsia caespitosa Distichlis spicata.*

Introduction

Ammoniacal-nitrogen (N) is a common constituent of mine waste residues, industrial wastewater, and agricultural runoff. The goal of this project was to assess the potential impact of elevated nitrogen levels on revegetation efforts planned for a Department of Energy (DOE) wetland mitigation site in Rifle, Colorado. Prior to wetland construction, elevated levels of both nitrate-N and ammoniacal-N were discovered in ground water at the project site. While high levels of nitrate-N are generally innocuous, excessive ammoniacal-N has been demonstrated to produce toxic effects in plants (Wong et al. 1983)(Vines and Wedding 1960) (Tiquia et al. 1996)(Lu 1998). Little information is available on wetland species response to high ammoniacal-N level, however (Surrency 1993). The purpose of this study was to evaluate plant response to elevated levels of ammoniacal-N in order to assist in the revegetation planning effort.

Site Description

The Rifle Wetland Mitigation Project is located at the site of a former uranium/vanadium processing facility along the Colorado River near Rifle, Colorado. The Department of Energy removed tailings piles located within the riparian zone and constructed a mitigation wetland as part of site restoration activities. Dominant vegetation on the site prior to wetland construction consisted of cattail (*Typha latifolia*), garrison creeping foxtail (*Alopecurus arundinaceus*), and salt cedar (*Tamarix ramosissima*). Site soils are of

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alluvial origin and sandy loam to loam in texture. Surface soils are alkaline (pH 8.0) and saline (EC 12.1-13.2 mmhos/cm) (MACTEC data). In contrast, ground water is slightly acidic to neutral (pH 6.5-7.0) (MACTEC Data). Ground water nutrient levels vary dramatically with concentric rings surrounding ammonium levels of 669 mg/L and nitrate levels of 1080 mg/L (MACTEC data). Ground water nutrient concentrations vary both annually and seasonally as nutrients are leached from the contamination plume. Surface water as diluted by high river flood stages ranges from 98 to 302 mg/L ammonium (MACTEC data).

Background on Ammoniacal-N Phytotoxicity

To understand ammoniacal-N induced phytoxicity, a basic understanding of nitrogen chemistry is required. Ammoniacal-N in aqueous solution is composed of the cation form termed "ammonium" (NH_4^+) and the uncharged form termed "ammonia" (NH_3). The pH and temperature parameters control the proportion of either form in solution at any given time. High pH and temperature favor the occurrence of NH_3. Under high pH and high temperature, NH_3 is highly volatile and can be removed from aquatic systems by mass transfer (Kadlec and Knight 1996). NH_3 can also be readily oxidized and result in significant oxygen consumption within wetland systems (Kadlec and Knight 1996). Ammoniacal-N, although generally a smaller proportion of N in solution, is the most available form to plant materials.

Ammoniacal-N has been reported as a plant toxin in several studies (Wong et al. 1983) (Vines and Wedding 1960) (Tiquia et al. 1996; Lu 1998). The toxicity of ammoniacal-N is usually attributed to the NH_3 form although conflicting reports occur in the literature (Bennett 1974) (Vines and Wedding 1960) (Gill and Reisenauer 1993). Symptoms of NH_3 toxicity include browning of roots, root death, and wilting of leaves(Bennett 1974). NH_4^+ toxicity symptoms reported in the literature include curling and drying at the leaf margins, chlorosis and necrosis of the laminae, depressed root growth, and reduced seed germination (Bennett 1974) (Cao, Glass et al. 1993; Tiquia, et al. 1996). Indirect affects of high ammoniacal levels are drought stress, frost stress, and pathogen invasion (Fangmeier et al. 1994). Factors affecting NO_3/ NH_4^+ uptake are pH, rooting zone temperature, rooting depth, mycorrhization, ion concentration, and the dominant type of nitrogen assimilation pathway of the plant species. Addition of potassium has been demonstrated to eliminate or reduce the toxic affects of NH_4^+ (Cao et al. 1993).

A substantial amount of literature exists on nitrogen levels in native and treatment wetlands. Native wetlands, due to the denitrification process, typically have ammoniacal-N levels less than 2 mg/L (Kadlec and Knight 1996). Average inflows into wetlands constructed for water treatment are 4 to 9 mg/L ammoniacal-N while animal feedlots in general have concentrations that range from 1 – 770 mg/L ammoniacal-N (Kadlec and Knight 1996). A study of a southern treatment wetland associated with a livestock facility indicated that nutrient concentrations of 160 to 170 mg/L ammoniacal-N were tolerable to giant bulrush but caused stress in cattail (Surrency 1993).

Horticultural research has also demonstrated adverse affects resulting from high applications of NH_4^+. Conifer nurseries generally consider levels of 100 – 150 ppm N to be sufficient for plant growth during rapid growth periods (Landis et al. 1989). Assuming a 1:1 nitrate:ammonium mix, the optimum NH_4^+ levels are between 50 and 75 ppm NH_4^+. Optimum levels of ammonia for black spruce (*Picea mariana*) production are listed as 50 ppm NH_4^+ (Landis et al. 1989). Chlorosis occurred in black spruce at 300 ppm and some mortality occurred at 500 ppm. Levels of 250 ppm nitrogen have been demonstrated to inhibit root growth in hardwood and dryland shrub species as well as alter root to shoot ratios (Lu 1998). The form of nitrogen for horticultural studies is not listed in all of the instances reviewed.

Several studies have been conducted which evaluated high NH_4^+ levels in immature composts (Wong et al. 1983; Tiquia et al. 1996). The relative seed germination of two vegetable species showed a significant negative correlation with increasing NH_4^+ concentration(Tiquia et al. 1996). These studies demonstrate that seed germination was reduced by greater than 50% in most species tested at NH_4^+ levels of 3.88 to 3.22 mg g-1 (approximately 3880 to 3220 ppm NH_4). Germination equivalent to controls did not occur until NH_4^+ was lowered to 500 ppm. These studies were conducted using relatively fast growing species (kale, cabbage, cucumber) which have an excellent ability to assimilate large amounts of nitrogen within a short time period.

A substantial amount of literature exists on vegetation exposure to atmospheric NH_3 levels resulting from air pollution (Fangmeier et al. 1994). NH_3 as atmospheric deposition produced visible leaf injuries (chorosis) at levels from 0.03 mg m-3 to 5.0 mg m-3. Survival rates of *Calluna vulgaris* seedlings were strongly reduced by levels of greater than 50% at levels of 0.053 mg m-3 exposed for 8 months. Frost hardiness was reduced by greater than 50% in *Pinus sylvestris* seedlings exposed to 0.15 mg m-3 for 21 weeks.

Methods

Three separate studies were conducted to evaluate species response at the germination, initial seedling growth, and mature seedling stages. The two treatment factors tested were site water dilution level and pH level. Site water was tested at five separate dilution levels (0%, 10%, 25%, 50% and 100%) in order to evaluate the response of plant materials to elevated NH_4^+ levels. The pH treatment consisted of three pH levels (6.5, 7.5, and 8.5) per dilution and was utilized to evaluate the response of plant materials to various NH_3 levels. All studies were conducted under controlled conditions and randomized block designs. Solutions for all studies were mixed in a similar manner.

Solution Preparation

Treatment solutions were created from water obtained at the Rifle project site (Table 1). Site water was shipped in sealed plastic containers with minimal head space. Site water was stored under refrigerated conditions (2-5° Celsius) until used. Base solutions containing 0% (control), 10%, 25%, 50% and 100% site water were created by diluting site water with deionized water and stored under refrigerated conditions. The control treatment solution (0%) consisted of deionized watered with the addition of a standard complete nutrient solution. Treatment solutions were adjusted to pH levels of 6.5, 7.5, and 8.5 prior to each watering period by titration with 1 M KOH or 1M H_3PO_4 using a Hanna Instruments 9025 pH meter and standard titration equipment. New treatment solutions were created prior to each watering session. NH_4^+ concentrations of stored site water were tested midway through the study. The concentrations presented in Table 1 denote NH_4^+ concentrations after five weeks of storage. It should be noted that concentrations at time of shipment were 597 and 616 mg/L representing a reduction of greater than 30% in site water between sampling periods. This reduction is most likely related to incorporation of oxygen and transformation to nitrate during the period of the study.

Table 1. Treatment solutions used in investigations.

Treatment #	Treatment Solution (% Site Water/pH)	NH_4^+ (mg/L)	Est. NH_3 (mg/L)
1	0%/6.5	75	0.076
2	0%/7.5	75	0.765
3	0%/8.5	75	7.650
4	10%/6.5	43	0.044
5	10%/7.5	43	0.439
6	10%/8.5	43	4.387
7	25%/6.5	109	0.111
8	25%/7.5	109	1.112
9	25%/8.5	109	11.12
10	50%/6.5	220	0.224
11	50%/7.5	220	2.244
12	50%/8.5	220	22.44
13	100%/6.5	406	0.414
14	100%/7.5	406	4.142
15	100%/8.5	406	41.417

Investigation 1

The purpose of Investigation 1 was to evaluate germination response to the treatment solutions. Alkali grass (*Puccinellia airoides*), inland saltgrass (*Distichlis spicata v. stricta*), Nebraska sedge (*Carex nebraskensis*), and tufted hairgrass (*Deschampsia caespitosa*) were seeded into 15 X 90mm petri dishes containing two sheets of Archer regular weight germination paper. Seeding rate was 30 or 50 based upon seed size and germination characteristics. Each species was exposed to the 15 treatment combinations described in Table 1. Levels of the site water dilution treatment consisted of 100%, 50%, 25%, 10%, and 0% site water. Solutions were applied at a rate of 10 mL per petri dish and rewatered with solutions as necessary to prevent drying. Seeds were maintained under growth chamber conditions of 25 day/20 night degree Celsius temperature regime with 14 hours daylight and allowed a fourteen day germination period. At the end of fourteen days, number of germinants was counted and root and shoot lengths were measured for seven individuals within each treatment. Final analysis consisted of the calculation of relative seed germination (number of germinants in treatment/number of germinants in control); relative root growth (mean root length in treatment/mean root length in control); and germination index = (%seed germination) X (% root growth)/100%.

Investigation 2

The purpose of Investigation 2 was to evaluate initial seedling growth response to treatment solutions as described above. Alkali grass (*Puccinellia airoides*), Nebraska sedge (*Carex nebraskensis*), tufted hairgrass (*Deschampsia caespitosa*), inland saltgrass (*Distichlis spicata v. stricta*), and bulrush (*Scirpus acutus*) were grown in sand culture under hydroponic growth chamber conditions. Each species was exposed to all 15 treatment combinations within three replicated 1" X 1" X 1" plastic cells per species. All species were seeded at 10 seeds per cell with exception to inland saltgrass that was seeded at 25 seeds per cell. Seedlings were maintained under growth chamber conditions of 25 day/20 night degree Celsius temperature regime with 14 hours daylight and allowed a fourteen-day germination period. All plants were watered from below to prevent soil disturbance. Due to poor initial germination, plastic covers were temporarily placed over the top of hydroponic trays to increase humidity levels and germination. Plants were thinned to five individuals to prevent competitive interactions and watered with solution when cell surfaces became dry. At the end of forty-five days, remaining germinants were counted, root and shoot lengths were measured for three individuals per cell, and plants were photodocumented. Analysis of Variance was used to evaluate overall differences in independent variables with differences judged significant at the $p<0.05$ level. The Kruskal-Wallis Test was utilized to compare treatment effects on individual species.

Investigation 3

The purpose of Investigation 3 was to evaluate mature seedling response to the treatment solutions described above. The study consisted of containerized plants placed in hydroponic trays and exposed to all 15 treatment combinations for a period of eight weeks under greenhouse conditions. Trays contained four plants per species of streambank willow (*Salix exigua*) and Freemont's cottonwood (*Populus fremontii*) as 10 cubic inch containerized material and cattail (*Typha latifolia*), bulrush, and Nebraska sedge as 4 cubic inch containerized material. All plants were originally grown within a peat-based potting media. Each tray representing one treatment solution (15 trays total) were randomly placed on a greenhouse table and periodically rotated to prevent environmental effects. Solutions were prepared as above and added to trays when solution levels dropped below drainage holes on the container. All trays were periodically drained and algae scrubbed from containers before re-watering. Shoots were harvested, separated, dried, and weighed at the end of eight weeks. Analysis of Variance was used to evaluate overall and within species differences in survival and shoot biomass with differences judged significant at the $p<0.05$ level. One outlier was identified and removed from the analysis. Relative shoot biomass was calculated and graphed. Plant color and phytotoxicity symptoms were photodocumented.

Results & Discussion

Investigation 1

Site water appears to inhibit germination and root elongation of certain species at various levels of the dilution-pH treatments while stimulating germination and root elongation at other levels. Tufted hairgrass and alkali grass are able to germinate and initiate growth under 100% site water conditions at all pH levels tested. Nebraska sedge appears to be sensitive to water which exceeds 25% site water at pH levels of 6.5 and 7.5 while germination and root elongation at pH 8.5 was similar to or exceeded the control at all dilution levels. Bulrush appears sensitive to site water levels of 10% or greater at the pH levels tested with exception to a pH level of 7.5, which stimulated both germination and root growth in dilutions up to 50%. Inland saltgrass failed to germinate and was eliminated from the analysis. Stimulatory effects in certain species are likely due to breakdown of seed coats and greater inbibation of seeds at increased pH or NH_4^+ levels. Conversely, excessive NH_4^+levels are likely responsible for seed or germinant mortality.

Investigation 2

Analysis of Variance was used to determine significant differences between the independent variables of percent survival, shoot length, root length, and root:shoot ratio due to the factors of dilution and pH treatments. The pH treatment was not judged significant and allowed for combining treatments and increasing the power of the ANOVA. Dilution treatment significantly affected all of the independent variables.

Dilution treatment significantly affected percent survival within the study. Increasing amounts of site water and subsequent higher ammoniacal-N levels resulted in sharp decreases in survival. Survival was reduced by half between the 0% and 10% site water dilutions and the 100% site water dilution (65 and 61% survival compared with 32% respectively). Analysis determined that percentage survival was statistically significant between dilution treatments. In comparing dilutions, the 50%, 25%, and 10% dilutions were similar but significantly different than the 100% site water solution while the 10% and control were again similar (Table 2).

Table 2. ANOVA for idependent variables in Investigation 2 (n=45).

Treatment Solution	Survival (%)	Mean Shoot Length (cm)	Mean Root Length (cm)	Root: Shoot Ratio
0% SW	65a	4.41a	1.67a	0.34a
10% SW	61ab	2.48b	1.35b	0.33a
25% SW	50b	1.61c	0.76c	0.33a
50% SW	48b	1.58c	0.33d	0.12b
100% SW	32c	0.62d	0.11d	0.06b

Similar letters form a group of means with no significant differences.

Figures 1-5 describe the relative survival, shoot length, root length, and root:shoot ratios as compared to the control. Root length, shoot length, and the root: shoot ratios were all statistically different ($p<0.01$) when compared across dilution treatments. In general, higher NH_4^+ levels resulted in stunted plants with inhibited root growth and a lower root:shoot ratio. These responses are in concurrence with NH_4^+ toxicity symptoms reported by others. While stunting of shoots was apparent at all levels in comparison to the control, the root:shoot ratio was similar between all treatments with 25% site water or less. The root: shoot ratio is probably the best predictor of potential survival and plant health. As such, the 25% dilution level containing approximately 100 ppm NH_4^+ provides the least risk to seeded species at the site.

Individual plant species were also compared to determine whether certain species are able to tolerate high levels of ammoniacal-N. Due to the low number of replicates and high variance, the Kruskal-Wallis Test was used to compare medians of alkali grass, tufted-hairgrass, and Nebraska sedge. Bulrush and inland saltgrass were excluded due to lack of sufficient data to perform analysis.

Ammoniacal-N levels did not significantly affect survival in Nebraska sedge and alkali grass. Nebraska sedge survival was, however, substantially lower in the 100% site water treatment than all other dilutions. Tufted hairgrass was significantly lower at the 100% site water level than all other dilutions. Survival in the 100% site water treatment was 63% of the control for tufted hairgrass. While insufficient data was available for bulrush and inland saltgrass due to poor germination, it should be noted that surviving germinants for these species occurred almost exclusively in the 0% to 25% site water treatments. Plant survival within the short time frame of the study under controlled conditions does not appear to be an important factor. It is important to consider that these plants did not experience water stress or high desiccation rates which may result in a much more significant effect under field conditions.

Shoot length was significantly affected by ammoniacal-N levels in alkali grass, tufted hairgrass, and Nebraska sedge. Tufted hairgrass appears most tolerant when this factor is considered with significant reductions in shoot biomass occurring only at the 100% site water level. Alkali grass shoots, in contrast, were reduced to 41% of the control at the 25% level. Nebraska sedge demonstrated an even greater sensitivity with shoot length reduced to 37% of the control at the 10% site water dilution. Shoot length indicates that only tufted hairgrass has limited tolerance to NH_4^+ levels experienced at the site.

Root length was also significantly affected by increasing levels of NH_4^+ in alkali grass, tufted hairgrass, and Nebraska sedge. Alkali grass and tufted hairgrass exhibit a similar tolerance with substantial reductions occurring at the 25% site water dilution level. Nebraska sedge experienced a reduction of 22% relative to the control at the 25% site water level. Root tips in several individual plants were noted as browning at the higher ammoniacal-N levels in contrast to white root tips in the control. This provides direct evidence of a phytotoxic effect of NH_3 within the site water.

Root:shoot ratios were significantly different for alkali grass and tufted hairgrass, but not for Nebraska sedge. Root: shoot ratios actually increased for alkali grass at the 10% and 25% site water treatment levels before dropping to 41% of the control at the highest site water treatment level. This is due primarily to strong reductions in shoot length at these two levels as opposed to increases in root length. This suggests that damage associated with high ammoniacal levels is occurring in shoots as opposed to roots. Nebraska sedge experiences a similar pattern although this was not judged significantly different by the Kruskal-Wallis Test. Tufted hairgrass root:shoot ratios were significantly reduced at the 50% dilution treatment level indicating substantial loss of root growth at approximately 200 ppm NH_4^+.

Several general observations of interest were noted during data collection. Of particular interest, algae heavily colonized sand cultures with 25% or less site water while the 50% and 100% site water cells contained little or not algae at the conclusion of the experiment. This provides further evidence that ammoniacal-N levels of greater than 200 ppm are strongly inhibitory to plant growth. Another observation was the browning of roots in certain species at higher levels of ammoniacal-N. Browned roots were assumed to be dead or experiencing dieback and correlated well with both reductions in the root:shoot ratios.

Another observation concerns the comparison of NH_4^+ levels between the site water and controls. Controls (0% site water) contained approximately 75 ppm NH_4^+ while the lowest dilution treatment (10% site water) contained approximately 20 ppm NH_4^+. Controls, however, consistently outperformed this treatment, which contained lower levels of the expected phytotoxin. This is explainable by the fact that the control represents a balanced solution where no required plant nutrients are limiting while the treatment solutions may have had other limiting nutritional factors such as nitrogen. As well, the literature suggests that some mineral nutrients may act to buffer the toxic effects of ammoniacal-N.

High ammoniacal-N levels in site water are responsible for a phytotoxic response at the early seedling stage as evidenced by reductions in root and shoot growth, browning of roots and an observed inhibitory effect on algae growth in treatments. Although this study did not experience significant reductions in seedling survival, it can be assumed that reduced root length would result in substantial mortality under field conditions. Tufted hairgrass and alkali grass demonstrated a limited tolerance to elevated NH_4^+ levels up to approximately 100 ppm NH_4^+ -N. The upper limits of tolerance for Nebraska sedge are between 40 and 100 ppm NH_4^+ -N. As mentioned previously, bulrush and inland saltgrass produced seedlings in only the control (100 ppm NH_4^+) and 10% site water dilutions (approximately 40 ppm NH_3) suggesting sensitivity at the seedling stage although this could not be statistically confirmed due to overall poor germination rates.

Investigation 3

Analysis of Variance was used to determine significant differences between the independent variables of percent survival and shoot biomass due to the factors of dilution and pH treatments. The pH treatments did not affect either survival or shoot biomass. As with Investigation 2, pH treatments were combined with dilution treatments to increase the power of the ANOVA. Percentage survival was not affected by dilution treatments. Increasing amounts of site water and subsequent higher ammoniacal-N levels did result in significantly reduced shoot biomass, however. The control (0% site water) was significantly different from all other dilution treatment levels while dilution levels of 10% and 25% were significantly different from the 100% site water level. The 50% dilution level was similar to straight site water and the 10% and 25% dilution levels.

Table 3. ANOVA for shoot biomass in Investigation 3* (n=15).

Treatment Solution	Shoot Biomass (g)
0% SW	2.82a
10% SW	1.72b
25% SW	1.90b
50% SW	1.30bc
100% SW	1.01c

*Similar letters form a group of means within which there are no statistically significant differences.

Individual plant species were compared using ANOVA. High ammoniacal-N levels significantly reduced the shoot biomass of streambank willow, Fremont cottonwood, and Nebraska Sedge. In general, we observed three types of responses by plants exposed to site water: 1) negative response associated with nutrient deficiency; 2) positive response associated with nutrient addition; and 3) phytotoxic response associated with elevated NH_4^+ levels.

Streambank willow shoot biomass was significantly reduced at the 50% and 100% site water treatments from all other treatments. Streambank willow at the highest treatment level was 30% of the relative control biomass and less than half of the next most productive treatment. Symptoms of toxicity observed on willow at the 50% and 100% dilution levels included a lack of rigidity in stems, elongated stems, leaf loss occurring from the oldest to youngest leaves, and chlorosis of leaves occurring towards the interior portions of the leaf surface. Subsequent to leaf loss, new buds formed on the stem axes as plants attempted to form new leaves. Although survival was not a significant variable for this treatment, death appeared imminent for plants in the 50% and 100% treatment levels. Streambank willow does, however, appear tolerant to NH4+ levels up to the 25% site water treatment (approximately 100 ppm NH_4^+).

Increasing levels of ammoniacal-N also significantly reduced Fremont cottonwood shoot biomass. The control achieved the greatest shoot biomass while 10% and 25% dilution treatments formed similar biomass as did the 25% and 50% and the 50% and 100% dilution treatments. Symptoms of toxicity were similar to the streambank willow. Cottonwood at high levels of ammoniacal-N exhibited lack of rigidity in stems, elongated stems, leaf loss occurring from the oldest to youngest leaves, and chlorosis of leaves occurring towards the interior portions of the leaf surface. As with streambank willow, survival was not a significant variable for the 100% dilution treatment but death did appear imminent. Assuming some loss of biomass is acceptable, cottonwood appears tolerant to NH_4^+ levels up to the 50% site water treatment level (approximately 200 ppm NH_4^+).

Shoot biomass of Nebraska sedge was significantly reduced by increasing amounts of ammoniacal-N. The control was significantly different from all other species while the 10% dilution level treatment was significantly different from the 100% treatment level. All other treatments (25%, 50%, and 100%) were similar. The relative shoot biomasses varied by 53% and 40% from control in the 10% and 100% treatments. Although this difference is significant, it is certainly not dramatic. The symptom of potential phytoxicity noticed on the 100% dilution treatment was death of older outside leaves on the plant. Given the minor loss of biomass between the 10% and 100% treatment level, Nebraska sedge appears tolerant to NH_4^+ levels up to the 50% site water treatment level (approximately 200 ppm NH_4^+).

Neither bulrush nor cattail were affected by increasing levels of ammoniacal-N at any dilution treatment level. Average shoot biomasses of both species were below the control suggesting some other form of nutrient deficiency. Both cattail and bulrush as mature seedlings appear to be capable of tolerating the highest levels of NH_4^+ -N tested in the study (approximately 400 ppm).

Several observations were made during the duration of the study. A substantial contrast in water use occurred during the study. Treatments containing elevated NH_4^+ levels slowed water use and exhibited drought symptoms at approximately four weeks into the study. A substantial accumulation of salts occurred on the 50 and 100% site water treatments. As well, moss colonies growing on the surface of the treatments containing high amounts of site water died. All of these provide evidence that elevated NH_3 levels resulted in a phytotoxic effect.

High ammoniacal-N levels resulted in decreased plant productivity for three of five wetland species as evidenced by low shoot biomass levels, chlorotic leaves, leaf loss, drought-like symptoms and moss mortality within containers. Significant mortality was not experienced by the study although death appeared imminent for streambank willow and Fremont cottonwood at NH_4^+ levels greater than 100 ppm and 200 ppm NH_4^+ respectively. Nebraska sedge seedlings demonstrated some tolerance with significant reductions only at the 400 ppm NH_4^+ level. In contrast, bulrush and cattail were not affected by elevated levels of ammoniacal-N although biomass was substantially reduced in all treatments when compared with the control. It is suggested that nutrient limitation was responsible for this contrast.

Conclusions

High levels of ammoniacal-N will directly impact revegetation efforts at the Rifle Wetland Project site. Since concentrations occur in concentric rings, however, certain areas may be able to be planted based upon individual species tolerance. The following conclusions and recommendations are based upon the results of this study:

- Seed germination and initial seedling growth were affected by high ammoniacal-N levels as evidenced by reductions in germination percentage, reductions in root and shoot growth, browning of roots and an observed inhibitory effect on algae growth in treatments. In consideration of the revegetation options, levels of 100 ppm NH_4^+ -N or less within an 18 inch rooting zone should be considered amenable to seeding of tufted hairgrass and alkali grass. At levels greater than 100 ppm NH_4^+, substantial reductions in shoot growth and stand sustainability can be expected. The upper limits of tolerance for Nebraska sedge are between 40 and 100 ppm NH_4^+ -N.

- Mature seedling growth was affected by high ammoniacal-N levels in three of five wetland species as evidenced by low shoot biomass levels, chlorotic leaves, leaf loss, drought-like symptoms and moss mortality within containers. Significant mortality was not observed although death appeared imminent for streambank willow and Fremont cottonwood at NH_4^+ levels greater than 100 ppm and 200 ppm respectively. Nebraska sedge seedlings demonstrated some tolerance with significant reductions only at the 400 ppm NH_4^+ level. In contrast, elevated levels of ammoniacal-N did not affect bulrush and cattail although biomass was substantially reduced in all treatments when compared with the control. In consideration of revegetation options, streambank willow may be planted in substrates exposed to ground water levels up to 100 ppm NH_4^+, Fremont cottonwood and Nebraska sedge may be planted in substrates exposed to ground water levels up to 200 ppm NH_4^+; and cattail and bulrush may be planted in substrates exposed to ground water levels up to 400 ppm NH_4^+.

- Species which reproduce primarily by vegetative means (i.e. – bulrush and cattail) should be used in high NH_4^+ areas (> 100 ppm NH_4^+) in order to avoid inhibition of seed germination.

- NH_3 level as influenced by the pH treatment did not influence results in Investigation 2 or 3. It is assumed that high temperatures (>20°C) may have resulted in the volatilization of NH_3, thus preventing fair consideration of this factor. This should be considered in transferring greenhouse scale studies to field conditions.

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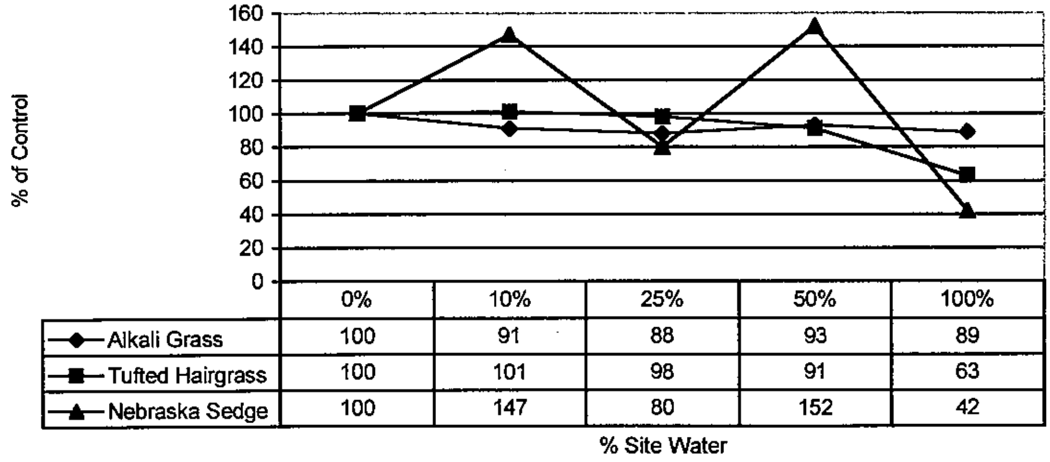
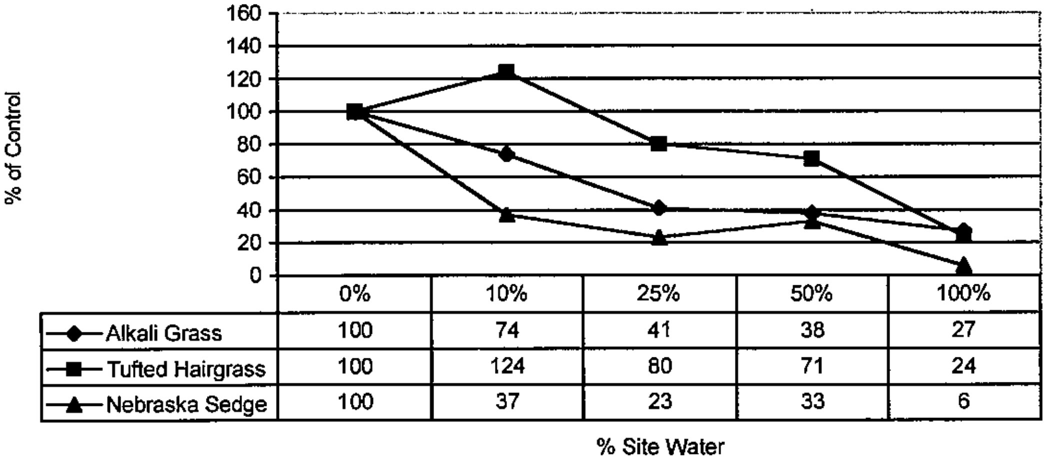
Figure 1. Relative Survival for Investigation 2



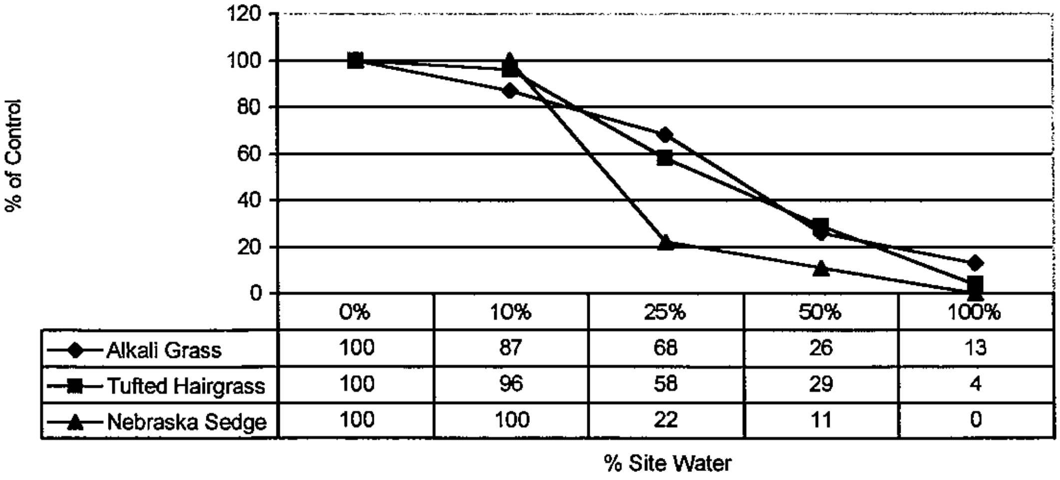
	0%	10%	25%	50%	100%
—◆— Alkali Grass	100	91	88	93	89
—■— Tufted Hairgrass	100	101	98	91	63
—▲— Nebraska Sedge	100	147	80	152	42

Figure 2. Relative Shoot Length for Investigation 2



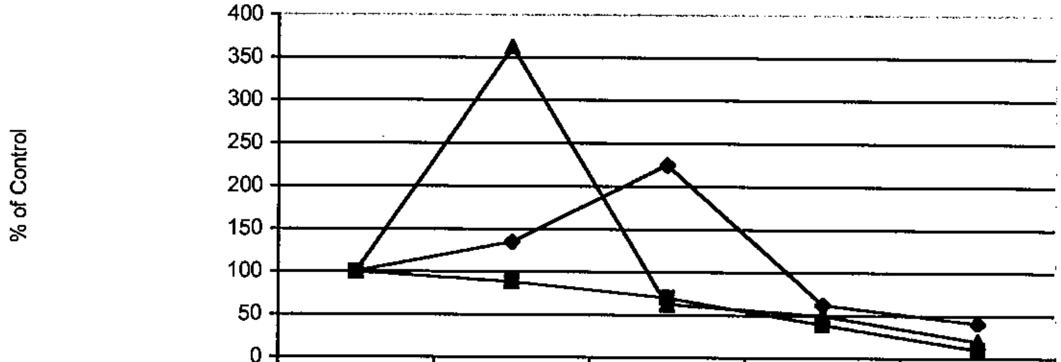
	0%	10%	25%	50%	100%
—◆— Alkali Grass	100	74	41	38	27
—■— Tufted Hairgrass	100	124	80	71	24
—▲— Nebraska Sedge	100	37	23	33	6

Figure 3: Relative Root Length for Investigation 2



	0%	10%	25%	50%	100%
—◆— Alkali Grass	100	87	68	26	13
—■— Tufted Hairgrass	100	96	58	29	4
—▲— Nebraska Sedge	100	100	22	11	0

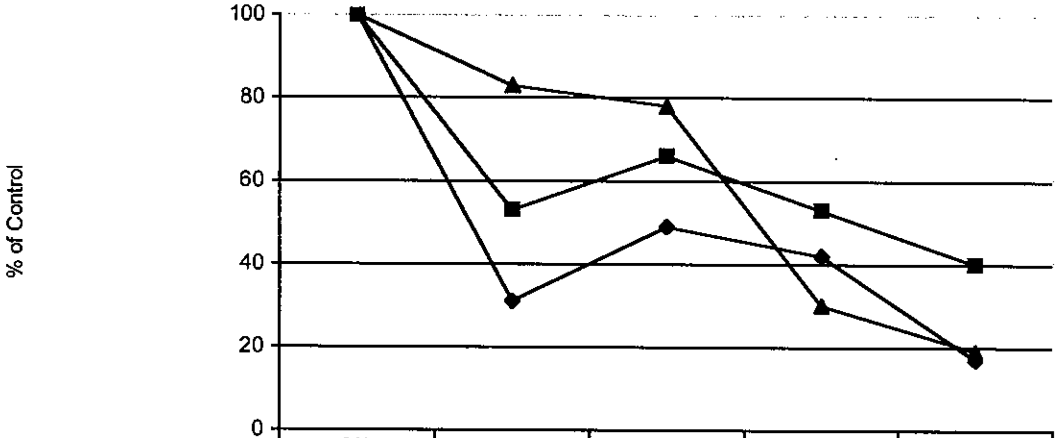
Figure 4: Relative Root:Shoot Ratios for Investigation 2



	0	10%	25%	50%	100%
—◆—Alkali Grass	100	135	225	62	41
—■—Tufted Hairgrass	100	88	70	39	10
—▲—Nebraska Sedge	100	362	62	50	20

% Site Water

Figure 5: Relative Shoot Biomass for Investigation 3



	0%	10%	25%	50%	100%
—◆—Cottonwood	100	31	49	42	17
—■—Nebraska Sedge	100	53	66	53	40
—▲—Willow	100	83	78	30	19

% Site Water