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Nitrate Concentration of Water in Hydroponic System Impacts Nitrogen Concentration of Wheatgrass Roots and Shoots Differently

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ABSTRACT

Nitrate is the most common groundwater contaminant. The objective of this experiment was to determine if *Triticum aestivum* (wheatgrass) is able to act as a phytoremediator of nitrates present in wastewater and how nitrogen (N) accumulation in wheatgrass is affected by the nitrate concentration in a hydroponic system. Wheatgrass was reared in six hydroponic units containing 0, 100, or 200 ppm added nitrate (n = 15 tanks for 100 and 200 ppm added nitrate and five for 0 ppm added nitrate control). Plants were grown for 12 days prior to harvest. The harvested shoots and roots were dried, ground, and analyzed for total nitrogen. Plants grown in 200 ppm nitrate solution contained a greater (9.9%; $P \leq 0.05$) concentration of nitrogen in the shoots than control plants with the 100 ppm nitrate solution being intermediate. Plants grown in 200 ppm nitrate solution contained a lower (16%; $P \leq 0.05$) concentration of nitrogen in the roots than control plants with the 100 ppm nitrate solution being intermediate. Nitrate and nitrate-N concentrations in the water were reduced to levels considered to be non-problematic for consumption by mature cattle for the 100 ppm treatment, but not the 200 ppm treatment.

KEY WORDS: wheatgrass, nitrate, nitrogen

INTRODUCTION

Nitrate regulation is important to both society and the aquaculture industry. Consumption of elevated levels of nitrates in drinking water has been linked to several health conditions including methemoglobinemia in infants and some forms of cancer (Weyer et al. 2001; Ward et al. 2005). Excessive nitrate intake has also been implicated as a cause of spontaneous abortions in animals (Manassaram et al. 2006). Nitrate in water often results from nonpoint sources such as agricultural and fertilizer (Loehr 1974; Keeney and Olson 1986) or point sources such as leaks from sewage treatment systems (Keeney and Olson 1986).

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Nitrate levels potentially affect human society as drinking water quantity and quality, as well as physical health are threatened (Goff 2004). High fertilizer runoffs from urban lawns and farmlands, septic systems, and livestock discharge are one of the main reasons for nitrate being the most common groundwater contaminant (Keeney and Olson 1986). Current regulations in the United States require that the Maximum Contaminant Level (MCL) for drinking water standards should not exceed concentrations of 10 ppm nitrate-N or 45 ppm nitrate. This sole standard for nitrate level was initially set to combat a condition of methemoglobinemia, commonly known as blue baby syndrome (Knobeloch et al. 2000). This occurs due to high liquid intake compared to body weight, which results in low acidity in the stomach allowing for bacterial conversion of nitrate to nitrite. The interaction between nitrite and hemoglobin then decreases the blood's capacity to carry oxygen. There are also equally serious, chronic conditions that are suspected to develop after long-term exposure to nitrate-contaminated water. Health effects include hypertrophy of the thyroid, 15 kinds of cancer, two kinds of birth defects, and hypertension (Anjana and Iqbal 2007).

The use of cyanobacteria (photosynthetic microorganisms) to biologically remove nitrate from groundwater has shown promise (Hu et al. 2000). The use of plants for the purpose of phytoremediation of nitrate-contaminated water may also be effective (Leba et al. 1999; Sundaralingam and Gnanavelrajah 2014). *Triticum aestivum* (wheatgrass) belongs to the Poaceae family and is a type of grass commonly grown in the temperate regions of Europe and the United States. The primary objective of this research is to determine the ability of wheatgrass to be used as a phytoremediator of nitrates present in aquaculture wastewater.

MATERIALS AND METHODS

Wheatgrass seeds were planted in individual hydroponic units situated atop individual aquarium tanks containing nitrate treatments of 200 ppm, 100 ppm, or 0 ppm nitrate (control). Replications consisted of five tanks per treatment, which contained six hydroponic cups per tank. Nitrate levels were achieved by dissolving $NaNO_3$ powder in the aquarium tanks and were monitored throughout the study using nitrate test strips. Water samples (50 ml) were collected for nitrate and nitrate-nitrogen analysis upon completion of the 12-day growing period. Water samples were frozen immediately following collection and stored for later analysis by Dairy One (Ithaca, NY, USA). After the 12-day growing period, wheatgrass was harvested and sorted by plant tissue (shoots or roots) from each treatment. Harvested plant materials were dried at 55 °C in a forced air oven for 48 h, then ground to pass a 1-mm screen in a sheer mill (Wiley Arthur H. Thomas Co., Philadelphia, PA) and stored for subsequent chemical analysis. Plant tissue dry matter was determined by drying overnight at 105 °C in a forced air oven. Dried plant materials were analyzed for N using an Elementar Vario Macro C:N analyzer (Elementar Americas, Inc., Mt. Laurel, NJ).

The GLIMMIX procedure of SAS (SAS Inst. Inc., Cary, NC, USA) was used for statistical analysis of DM and N concentration of shoots and roots. The model included the main effect of N concentration on the dependent variables root and shoot percent N. Least Square means were estimated using the LSMEANS statement and when significant effects were detected in the model ($P \leq 0.05$), the LINES option with the TUKEY adjustment was used for mean separation. Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA) was used for statistical analysis of 12-day water nitrate reduction. An unpaired t test was used and $P \leq 0.05$ was considered significant.

RESULTS

The effects of hydroponically grown wheatgrass on water nitrate and nitrate-N concentrations after 12 days of plant growth are shown in Table 1. Nitrate concentrations decreased in water used for the hydroponic system over the 12-day period of wheatgrass growth. There was no difference in the reduction in nitrate concentrations over a 12-day period between the 100 and 200 ppm treatments ($P = 0.27$). Water nitrate and nitrate-N concentrations of the 200 ppm treatment remained two-fold greater than that of the 100 ppm treatment over the 12-day period.

Table 1. Water nitrate and nitrogen concentrations (ppm) and reductions (%) for water containing 0, 100, or 200 ppm Nitrate before (day 0) and after (day 12) of hydroponic wheatgrass growth.

Treatments Nitrate, ppm	Nitrates, ppm			Nitrogen, ppm
	Day 0	Day 12	% Reduction	Day 12
0	0	0^{c}	-	-
100	100	73^{b}	27^{a}	17^{b}
200	200	161^{a}	19.5^{a}	37^{a}

$^{a, b, c}$ Within a column, means with different superscripts differ by ANOVA ($P \leq 0.05$). Mean separations were performed with the LINES (TUKEY) option of the LS MEANS statement in PROC GLIMMIX.

The accumulation of total N in roots and shoots of wheatgrass grown in a hydroponic system treated with 0, 100, or 200 ppm nitrate is shown in Figure 1. Accumulation of N in roots decreased with increasing water-nitrate concentrations and was less in the 200 ppm treatment than the 0 ppm control. Accumulation of N in shoots increased with increasing water nitrate concentrations and was greater in the 200 ppm treatment than the 0 ppm control. Accumulation of N in wheatgrass shoots and roots grown in 100 ppm nitrate was intermediate to the 0 ppm and 200 ppm treatments.

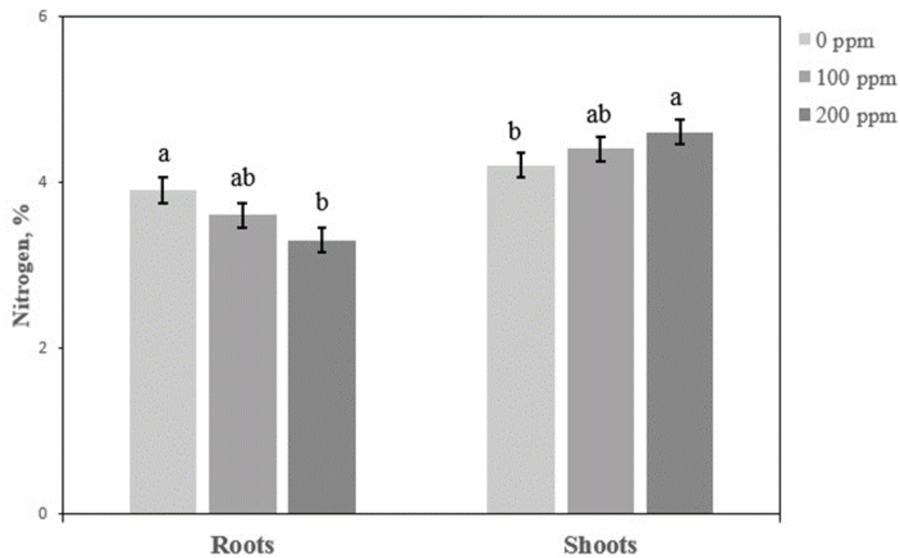

Figure 1. Plant tissue nitrogen concentration of wheatgrass grown hydroponically in water containing 0, 100, or 200 ppm nitrate.
Treatment x plant part interaction, $P = 0.01$
[a,b,c] Bars with common letter do not differ, ($P \leq 0.05$)

CONCLUSION AND DISCUSSION

A plant's capability to remediate the environment is achieved either by storing the chemicals in their structural components, using their own metabolic processes to produce a chemical that is less harmful, or by transforming harmful chemicals into trace amounts of gas. Phytoremediation is the use of living plants, particularly their structural components such as their roots and metabolic processes, to rehabilitate contaminated soil, water, or air (Macek et al. 2000). Phytoremediation is a relatively inexpensive method with estimates of $0.60 - $6.00 per 1000 gallons of remediated water (Macek et al. 2000). Some limitations of this method include the depth of the roots and the tolerance of the plant to the contaminant. A particular type of phytoremediation is called phytodegradation, where the internal and external metabolic processes of the plant drive the degradation of organic contaminants, and as the plant grows, they become incorporated into plant tissues or further degraded. The basic concept of a hydroponic system is that of growing a plant in water containing essential nutrients, such as potassium and nitrogen for growth, in the absence of soil.

Nitrate accumulation can occur in the shoots and roots and tends to accumulate in the initial growth period (Anjana and Iqbal 2007). Besides nitrate availability in the soil or water, the plant's inherent metabolism also determines accumulation rates. The primary objective of this research was to determine if the total N concentration of wheatgrass roots and shoots increases when grown hydroponically in 0, 100, and 200 ppm lab-prepared nitrate solutions. The percent nitrogen of 0 and 200 ppm nitrate treatments differed ($P < 0.05$). The plants grown in a 200 ppm nitrate water source had 3.16% N in roots and 4.89% N in the shoots. The control group with 0 ppm nitrates produced wheatgrass with 2.51% N in the roots and 4.35% N in the shoots.

The hydroponic units treated with 200 ppm nitrates had greater percent nitrogen presence in the roots and shoots when compared to the 0 ppm control. The fact that the shoots had a higher percent of nitrogen may or may not pose a potential problem regarding further use of the harvested crop as a food, feed, or cover crop. Further investigation is needed in order to accurately determine what form of nitrogen is present in the shoots. In the event that after nitrate phytoremediation the harvested wheatgrass is overly contaminated with nitrate, then the popular method of ensiling the crop, where anaerobic bacteria decompose the harmful nitrate to safer nitrogen forms, can be used to allow the making of forage. Wheatgrass could still provide a nutritional benefit to humans, provided that after its phytoremediation role in aquaculture wastewater, the crop is still safe for consumption. Wheatgrass may also be used as an alternative source of livestock feed because of its resulting higher nitrogen level if these concentrations translate into increased crude protein. In addition to the possible benefit of increased crude protein, nitrate inclusion in cattle diets has been shown to reduce enteric methane emissions by as much as 16% (Van Zijderveld et al. 2011). Methane produced by cattle can represent as much as 15% loss in metabolizable energy intake by the animal and is a potent energy trapping greenhouse gas. Mitigation efforts that do not result in negative effects on digestibility of the diet and overall animal production are of great interest. The effects of dietary nitrate on rumen physiology may be the result of changes in microbial population. Shifts in rumen-microbial populations have been observed in cattle consuming diets supplemented with nitrate-N (Lin et al. 2013).

A diet that provides essential nitrogen allows for not only higher crude protein for livestock, but wheatgrass itself could serve as a nitrogen-rich cover crop. The wheatgrass plant might be able to help the aquaculture industry relative to water remediation and also the agriculture industry in its recycling of the crop to be used as forage or cover crop. In terms of the control treatment with 0 ppm nitrates, a similar pattern of the shoots containing a higher nitrogen presence than the roots was observed. This pattern makes sense because the leaves contain chlorophyll, of which nitrogen is an important component. As for the question behind the still significant nitrogen presence in the control group with initial 0 ppm nitrate, the nitrogen might have come from a combination of the inherent nitrogen in the seed itself and its growing medium that is made of natural coconut fibers (cocotek cups lined the hydroponic cups). Wheatgrass plants grown from a 200 ppm nitrate source produced shoots and roots containing significantly more nitrogen.

Nitrate-N concentrations of water used to grow hydroponic wheatgrass decreased over a 12-day period of growth. While wheatgrass is able to phytoremediate water containing 100 ppm Nitrate-N to safe levels (below 80 ppm), it was not able to do so at the 200 ppm concentration. The hypothesis that the wheatgrass roots and shoots would contain greater N concentrations when grown in 200 ppm nitrate solutions as compared to those grown in 0 ppm nitrate was correct. The greater concentration of total N in the shoots than in the roots raises a question of whether the crop is still suited for consumption. Since nitrate-N was not measured in plant tissue, further investigation is needed to identify what form of nitrogen is actually present in those plant structures, the harmful nitrate or elemental N form, before suitable uses of the crop can be recommended. Nitrate does have a significant effect on the N composition of the wheatgrass plant, most particularly, its shoots.

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