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Uptake and translocation of sulfamethazine by alfalfa grown under hydroponic conditions

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ABSTRACT

Antibiotics are routinely used in intensive animal agriculture operations collectively known as Concentrated Animal Feed Operations (CAFO) which include dairy, poultry and swine farms. Wastewater generated by CAFOs often contains low levels of antibiotics and is typically managed in an anaerobic lagoon. The objective of this research is to investigate the uptake and fate of aqueous sulfamethazine (SMN) antibiotic by alfalfa (*Medicago sativa*) grass grown under hydroponic conditions. Uptake studies were conducted using hydroponically grown alfalfa in a commercially available nutrient solution supplemented with 10 mg/L of SMN antibiotic. Analysis of alfalfa sap, root zone, middle one-third, and top portion of the foliage showed varying uptake rate and translocation of SMN. The highest average amount of SMN (8.58 μg/kg) was detected in the root zone, followed by the top portion (1.89 μg/kg), middle one-third (1.30 μg/kg), and sap (0.38 μg/kg) samples, indicating a clear distribution of SMN within the sampled regions. The ultraviolet (UV) spectra of parent SMN and translocated SMN identified in different parts of the plant present the possibility of metabolization during the uptake process. Uptake of SMN using alfalfa grown under hydroponic conditions has potential as a promising remediation technology for removal of similar antibiotics from wastewater lagoons.

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Introduction

Antibiotics used in intensive animal agricultural operations end up in a lagoon. These lagoons may be susceptible to flooding, potentially impacting surrounding watersheds. Antibiotics resist degradation under anaerobic conditions, but their removal prior to releasing treated water into the environment is important for microbial resistance management and to prevent environmental contamination. The major classes of antimicrobials including sulfonamides, macrolides, aminoglycosides, tetracyclines, and ionophores are routinely used in animal agriculture operations (Thiele-Bruhn, 2003). Various studies documented the use of pharmaceuticals in intensive animal agriculture practices and their subsequent occurrence in the environment (Daughton and Ternes, 1999; Kolpin et al., 2002; Campagnolo et al., 2002; Boxall et al., 2003, 2004). Once released into the environment, antibiotics are resistant to biodegradation and have a tendency to accumulate in the environmental matrices, and even at low concentration can adversely affect the aquatic and terrestrial environment (Homem and Santos, 2011). Restricting the usage of antimicrobials is one of the many ways to prevent the entry of antimicrobials in the environment. In intensive animal agriculture operations however, such options may not be economically feasible; especially in the

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United States where a large percentage (nearly one-third) of antimicrobials are being used at a sub-therapeutic level as a growth promoter (Levy, 2002). Although the occurrence of antimicrobials in the environment has been extensively studied, relatively few authors have investigated remediation protocols for their effective removal from the environment.

The studies that consider plant uptake of antimicrobials (Forni et al., 2002; Migliore et al., 2003; Boxall et al., 2006; Dolliver et al., 2007) do so from the viewpoint of soil media and focus on the remediation of land applied manure containing antibiotics. This approach may not be effective because of the heterogeneity of the soil and the complex interaction between manure, soil and antimicrobials. Antimicrobials tend to sorb on to the soil material and large amounts may remain in the sorbed phase, particularly in soils with substantial organic material and clay fractions (Kurwadkar et al., 2007). The sorbed antimicrobials may not be readily available for plant uptake and as such, the soil based phytoremediation approach may not be an effective management approach.

Removal of antibiotics using plant is a viable choice for antibiotics remediation if sufficient time is allowed for plant acclimatization and contaminant degradation (Salt et al., 1998; Singh and Jain, 2003; Aslund et al., 2007; Michelini et al., 2014). In uptake and translocation process, the plants can extract, detoxify, and/or sequester toxic antibiotics from Concentrated Animal Feed Operations (CAFO) wastewater, which may contain low levels of antibiotics. Lately this approach has emerged as a promising strategy for *in-situ* removal of numerous soil contaminants (Singh and Jain, 2003; Aslund et al., 2007; Kubiak et al., 2012; Favas et al., 2013). Hydroponic systems are a promising area for plant growth as they assure effective management of water, energy, and cost in confined spaces (Rius-Rui et al., 2014). A hydroponic culture constitutes a soil-less plant model system, which has been proven to allow uptake of the target compound with no interference from the soil matrix. Several authors have attempted hydroponic culture for remediating a variety of environmental pollutants (Kang et al., 2012; Rius-Rui et al., 2014; Das et al., 2014). Researchers have demonstrated the removal of pharmaceuticals found in biosolids and wastewater using hydroponically grown cabbage (*Brassica rapa* var. *pekinensis*) and a rapidly reproducing, specialized strain of the same species (Wisconsin Fast Plants) (Herklotz et al., 2010). The authors were able to detect all four of the tested pharmaceuticals in roots and leaves of the plants, as well as the stems of the Wisconsin Fast Plants. Two of the pharmaceuticals, carbamazepine and salbutamol studied by these authors, were also detected in the seed pods produced by the Wisconsin Fast Plants. The authors attributed the systemic distribution to symplastic uptake of pharmaceuticals as they cross the plant's epidermal tissue.

Given these and other results, the possibility of other fast growing plants sequestering a pharmaceutical from solution cannot be overlooked. For this research, alfalfa was chosen as a contaminant-tolerant, hydroponically-amenable plant that increases biomass reasonably quickly and requires irrigation or high rainfall to grow well (Flocco et al., 2002). Alfalfa is a perennial plant, which belongs to the Leguminosae (pea) family and bears abundant root systems, which has shown to remediate or tolerate various environmental pollutants (Bonfranceschi et al., 2009; Carrasco-Gil et al., 2013; Funes-Collado et al., 2013). Sulfamethazine (SMN) was selected as an antimicrobial for testing due to its widespread use in the beef, swine and poultry industries (Huang et al., 2011). Recently SMN has become a priority pollutant primarily because of its widespread detection in soil, surface water, drinking water and variety of agricultural produce (Dolliver et al., 2007; Hu et al., 2010; Huang et al., 2011; Awad et al., 2014). Furthermore, given the low molecular weight coupled with low sorption in soil media, SMN can be a prime candidate for plant uptake (Kurwadkar et al., 2007). The objective of this research is to demonstrate whether SMN can be taken up from a nutrient solution by hydroponically grown alfalfa in a greenhouse environment.

1. Materials and methods

1.1. Chemicals

SMN (CAS#1981-58-4; Assay ≥ 98%) was purchased from Sigma Aldrich, St. Louis, MO, USA. The nutrient solution for the hydroponic system was purchased from General Hydroponics, Sebastopol, CA, USA. The nutrient solution was primarily a performance pack and consisted of several formulations of nutrient solution. It was administered as recommended by General Hydroponics. HPLC grade acetonitrile, ammonium acetate, and glacial acetic acid were purchased from Fisher Scientific (Pittsburgh, PA). A 1 g/L stock standard solution of SMN was prepared in nano-pure water (18.2 MΩ/cm at 25°C) and briefly stored in a refrigerator at 4°C prior to its use in the experiment. A sub-stock of 10 mg/L was used for studying the uptake of SMN by alfalfa.

1.2. Experimental design

Alfalfa was grown from seedlings using the commercially available hydroponic system. The fully automated system consists of an ECO 185 submersible pump equipped with an oil-free motor, filter, Eco-Air 4-air pump, and four high-output air stones (Fig. 1). The system circulates highly oxygenated bottom water which accelerates plant growth. Within a week, the seeds were sprouted and within two weeks, the alfalfa showed significant growth. The nutrients were fed according to the prescribed feeding schedule recommended by General Hydroponics. The nutrient losses were compensated by completely replenishing the entire nutrient solution every week. Once the plants were approximately one foot tall, a representative blank sample was procured from all corners of the hydroponic system.

Prior to changing the nutrient solution with an antibiotic solution, the entire system was thoroughly cleaned. The plants, including roots, were thoroughly rinsed and re-grown in 15 gal of a nutrient solution containing 10 mg/L of SMN. The same nutrient feeding schedule was followed as during the growth phase. Water loss due to transpiration was compensated with nutrient solution containing the antibiotic dose. The dose of 10 mg/L of SMN was based on a screening-level phytotoxicity study which demonstrated that alfalfa exposed to concentration greater than 10 mg/L over 5 days experienced adversely affected germination, total length, root length and shoot length

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