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Abstract
Atrazine, a broad-leaf herbicide, has been used widely to control weeds in corn and other crops for several decades and its extensive used has led to widespread contamination of soils and water bodies. Phytoremediation with switchgrass and other native prairie grasses is one strategy that has been suggested to lessen the impact of atrazine in the environment. The goal of this study is to characterize: (1) the uptake of atrazine into above-ground switchgrass biomass; and (2) the degradation and transformation of atrazine over time. A fate study was performed using mature switchgrass columns treated with an artificially-created agricultural runoff containing 16 ppm atrazine. Soil samples and above-ground biomass samples were taken from each column and analyzed for the presence of atrazine and its chlorinated metabolites. Levels of atrazine in both soil and plant material were detectable through the first 2 weeks of the experiment but were below the limit of detection by Day 21. Levels of deethylatrazine (DEA) and didealkylatrazine (DDA) were detected in soil and plant tissue intermittently over the course of the study, deisopropylatrazine (DIA) was not detected at any time point. A radiolabel study using [^{14}C]atrazine was undertaken to observe uptake and degradation of atrazine with more sensitivity. Switchgrass columns were treated with a 4 ppm atrazine solution, and above-ground biomass samples were collected and analyzed using HPLC and liquid scintillation counting. Atrazine, DEA, and DIA were detected as soon as 1 d following treatment. Two other metabolites, DDA and cyanuric acid, were detected at later time points, while hydroxyatrazine was not detected at all. The percentage of atrazine was observed to decrease over the course of the study while the percentages of the metabolites increased. Switchgrass plants appeared to exhibit a threshold in regard to the amount of atrazine taken up by the plants; levels of atrazine in leaf material peaked between Days 3 and 4 in both studies.

Keywords
Atrazine; Phytoremediation; Switchgrass; Metabolites

Disciplines
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Comments
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Abstract

Atrazine, a broad-leaf herbicide, has been used widely to control weeds in corn and other crops for several decades and its extensive used has led to widespread contamination of soils and water bodies. Phytoremediation with switchgrass and other native prairie grasses is one strategy that has been suggested to lessen the impact of atrazine in the environment. The goal of this study is to characterize: 1) the uptake of atrazine into above-ground switchgrass biomass; and 2) the degradation and transformation of atrazine over time. A fate study was performed using mature switchgrass columns treated with an artificially-created agricultural runoff containing 16 ppm atrazine. Soil samples and above-ground biomass samples were taken from each column and analyzed for the presence of atrazine and its chlorinated metabolites. Levels of atrazine in both soil and plant material were detectable through the first two weeks of the experiment but were below the limit of detection by Day 21. Levels of deethylatrazine (DEA) and didealkylatrazine (DDA) were detected in soil and plant tissue intermittently over the course of the study, deisopropylatrazine (DIA) was not detected at any time point. A radiolabel study using [^{14}C]atrazine was undertaken to observe uptake and degradation of atrazine with more sensitivity. Switchgrass columns were treated with a 4 ppm atrazine solution, and above-ground biomass samples were collected and analyzed using HPLC and liquid scintillation counting. Atrazine, DEA, and DIA were detected as soon as one day following treatment. Two other metabolites, DDA and cyanuric acid, were detected at later time points, while hydroxyatrazine was not detected at all. The percentage of atrazine was observed to decrease over the course of the study while the percentages of the metabolites increased. Switchgrass plants appeared to exhibit a threshold in regard to the amount of atrazine taken up by the plants; levels of atrazine in leaf material peaked between Days 3 and 4 in both studies.

Keywords: Atrazine Phytoremediation Switchgrass Metabolites

1. Introduction

Control of broadleaf weeds in corn, sorghum, and sugarcane crops can be achieved through a reversible inhibition of photosystem II by the triazine herbicide atrazine (Henderson *et al.* 2007, Kruger *et al.* 1993, Shimabukuro and Swanson 1969). With 51 million pounds of atrazine applied across 18 states in 2010 and 6.8 million pounds applied in Iowa alone, it is one of the most widely used herbicides in the agricultural industry (NASS 2011). Widespread usage has resulted in contamination of both ground water and surface water sources by atrazine and its metabolites. It has been estimated that as many as 2,700 community water source wells and 214,000 private wells are contaminated with atrazine (USEPA 1990)., Atrazine was found to be present in all 129 samples from 75 Midwestern rivers and streams in 1998 (Battaglin *et al.* 2000). In 2003, the United States Environmental Protection Agency implemented an atrazine monitoring program in which approximately 30 community water systems in 10 states were required to monitor for the presence of atrazine in drinking water. The most recent data from 2011 found atrazine to be above the limit of detection in 3249 of 3527 raw water samples. Concentrations of the samples ranged from 0.05 ppb to 38.6 ppb (mean: 1.1 ppb; median 0.55 ppb) (USEPA 2012).

Agricultural fields are a major source of atrazine contamination. As much as 5% of the applied atrazine may be lost from a field through surface water runoff (Mersie *et al*. 2006). As a result, levels of atrazine in local waterways surrounding agricultural settings may exceed the Maximum Containment Level of 3 parts per billion (ppb) for drinking water set by the Environmental Protection Agency (Southwick *et al*. 1992, Southwick *et al*. 1995, USEPA 2009). Many strategies have been employed in an attempt to mitigate the concentration of atrazine

reaching ground and surface water bodies. Phytoremediation, a well-researched method, uses plants to degrade, sequester, or otherwise neutralize organic or inorganic contaminants in soil and water.

Native prairie grasses are commonly used in phytoremediation strategies. Their extensive fibrous root system can penetrate up to ten feet below the surface and can result in a greater surface area than other vegetation (Aprill and Sims 1990). Switchgrass (*Panicum virgatum*) is a native prairie grass that is found growing naturally in most of the United States and can survive many extreme weather conditions, pH levels, and various soil types (NRCS 2009). Phytoremediation studies have shown that switchgrass, alone or in combination with other native prairie grasses, is capable of removing atrazine from the environment. Stands of switchgrass in combination with other native prairie grasses can reduce atrazine in leachate by 43% as well as promote degradation in the rhizosphere (Belden and Coats 2004). A mass balance study, using a mix of three prairie grasses including switchgrass, showed that atrazine and its metabolites were distributed equally between leaf and root tissue (Henderson *et al.* 2007). More recent research used a microbe-free environment to show that switchgrass is capable of taking up and degrading atrazine into several metabolites (Murphy and Coats 2011). That study also showed that significantly more degradation occurred in soil as a result of the presence of switchgrass when compared with natural degradation such as chemical or photolytic degradation (Murphy and Coats 2011). Finally, another study looked at the bioremediation potential of five different grasses and reported that switchgrass treatments facilitated the most degradation of atrazine in the soil, with more than 80% of the atrazine being degraded into metabolites (Lin *et al.* 2008).

In preparation for a large-scale phytoremediation study using switchgrass to remediate atrazine in an agricultural setting, it is imperative that preliminary studies be conducted to

anticipate forthcoming results from this larger study. The goal of the preliminary fate study is to characterize the uptake, degradation, and fate of atrazine and possible metabolites in a phytoremediation setting similar to one that could be used in an agricultural setting. To accomplish these objectives, atrazine was applied to soil columns with switchgrass that had been established for three years and the fate was monitored. At the end of 21 days, soil and above-ground plant biomass were evaluated for quantification and identification of atrazine and its chlorinated metabolites DEA, DIA, and DDA (Figure 1).

Following the completion of the preliminary fate study, a second study was performed using radiolabeled [^{14}C]atrazine in simulated surface water runoff to track atrazine as it was degraded into a variety of metabolites with more accuracy than was previously possible, in an attempt to observe trends in the fate of atrazine instead of only observing intermittent peaks of metabolites. Additionally, this study tries to determine the presence of hydroxyatrazine, which was not detected in the above study, but has been detected in switchgrass residues in other studies (Henderson *et al*. 2007, Lin *et al*. 2008).

2. Methods

2.1 Preliminary Fate Study

2.1.1 Experimental Design

Twenty-seven columns were constructed from PVC pipe (76 cm x 20 cm) and placed in a greenhouse. Each column was filled with soil collected from an agricultural field in Clarke County, Iowa and amended with potting soil. The pH and organic matter of the amended soil were determined to be 7.20 and 9.6%, respectively, and soil composition was determined to be: sand 39.90%, silt 37.19%, clay 22.91%. Switchgrass seeds (Cave-in-rock variety) were planted in each column in the fall of 2006 at a density of 10 plants per cm^2 soil surface. Greenhouse

conditions for the experiment were 27±2°C, 16:8 L:D cycle for March-October and 4.5±2°C, 16:8 L:D cycle for October-March. Prior to application of atrazine, the switchgrass plants were grown for a period of 30 months. These columns had previously had atrazine applied, however, no atrazine or metabolites persisted in the soil or switchgrass tissues after 21 days and more than 11 months elapsed between the end of that study and the beginning of this preliminary fate study. On Days 0, 3, and 6, 360 ml of water containing 16 µg/ml (ppm) atrazine (provided in-kind by Syngenta) was applied directly to the soil and allowed to permeate the columns; no leaching of the solution was observed. Although this is a much higher concentration than is typically found in the environment, it was theorized that the higher concentrations of atrazine would result in higher metabolite concentrations to allow better visualization of degradative products. The repeated application 3 days apart was an attempt to simulate multiple runoff evens in a short period of time. Two soil samples and one above-ground biomass sample were taken from each column immediately after application on Day 0. Additional samples were collected on Days 1, 2, 4, 5, 7, 8, 11, 14, and 21.

2.1.2 Extraction of above-ground plant biomass

On each sampling day, three randomly selected columns were used for extraction samples. Above-ground switchgrass biomass was cut off at soil level. Three 10-g samples per column were weighed out and used for extraction. Each 10-g sample was rinsed with water, cut into pieces that were approximately two centimeters in length, and ground with a mortar and pestle in 60 mL of ethyl acetate for 10 minutes. The solvent was decanted off through a filter containing 15 g of sodium sulfate to absorb any water contained in the sample. The above procedure was repeated for a total of three times. The solvent extract was then placed in a turbovap evaporator and evaporated with nitrogen to a final volume of 1 mL. The extract was

quantitatively transferred into a syringe with a 0.45 μm micropore filter attached. The extract was passed through the filter into a volumetric flask to a total volume of 5 mL. Two milliliters of this was then pipetted into a gas chromatograph (GC) vial, and kept at -20°C until analysis.

2.1.3 Extraction of soil

Two 20-g samples of soil were collected from the top 15 cm of each column per extraction day. Soil was collected in French square bottles, and root and organic debris were removed from each sample. Sixty milliliters of ethyl acetate was added to each bottle and then was mechanically shaken at 300 rpm in a horizontal position for 20 minutes. The solvent was decanted off through a filter containing 15 g of sodium sulfate and the samples were processed in the same manner as the above-ground biomass samples. This procedure was repeated for a total of three times for each sample.

2.1.4 Analysis for parent and metabolic compounds

Extracts for both above-ground plant biomass and soil were analyzed on a Varian 3400 GC equipped with a Varian 8100 auto sampler and a nitrogen phosphorus detector (NPD). The column was a DB5 (5% phenyl-methylpolysiloxane nonpolar stationary phase), 0.25 μm film thickness, 30 m in length, 0.25 mm ID (J&W Scientific). GC operating conditions were as follows: oven parameters, 80°C hold for 2 min, increase 8°C/min to 190°C hold for 4 min, increase 6.5°C/min to 230°C hold for 0.25 min; inlet, splitless mode, 220°C; carrier gas, ultra-high purity helium, flow 30 mL/min; detector, 300°C, hydrogen flow 4.25 mL/min, air flow 175 mL/min. The retention times for the ethyl acetate solvent, DDA, DIA, DEA, and ATR were 2.92, 18.78, 20.23, 20.73, and 22.43 min, respectively. Peak areas were integrated using Peak Simple (SRI Inc., Menlo Park, CA, USA).

2.2 Radiolabel Study

2.2.1 Experimental design

Five of the previously constructed columns were utilized in this experiment. There was a period of 18 months between the end of the prior preliminary fate study and the start of the radiolabel study. Switchgrass plants were grown under the conditions described above throughout the entirety of the radiolabel study. On Day 0, four columns were treated with 200 ml of water containing 4 ppm atrazine (provided in-kind by Syngenta) representing agricultural runoff by applying the solution directly to the soil; no leaching occured. The concentration of atrazine applied in the radiolabel study was reduced to 4 ppm because it was hypothesized that 16 ppm used in the fate study may have stressed the plants, thus reducing their capability to take up and degrade atrazine. By decreasing the amount of atrazine applied in the radiolabel study, it was theorized that the plants would be less stressed. Additionally, a 4 ppm solution is a more realistic concentration that has been observed in a couple runoff studies (Hall 1974, Ritter *et al.* 1974). Each 200 ml solution was spiked with approximately 18 million disintegrations per minute (dpm) of [^{14}C]atrazine (specific activity = 21,806 dpm/µg atrazine) (provided in-kind by Syngenta) . One column was treated with only 200 ml of distilled water to act as an untreated control.

2.2.2 Processing of switchgrass material

On Days 1, 3, 5, and 7 following treatment with atrazine, two above-ground biomass samples per column were collected for extraction by cutting the stem off at the soil level. Switchgrass biomass samples were weighed and the entire above-ground biomass was processed as described above, using 30 ml of ethyl acetate instead of 60 ml. Following the quantitative transfer through the syringe, samples were concentrated to 1 ml and placed in an HPLC vial.

2.2.3 Analysis of samples

A 100-µl sample of both plant and sand extracts were analyzed using high-performance liquid chromatography (HPLC). A Hewlett-Packard 1100 series HPLC equipped with an autosampler was used to separate the metabolites and parent compound. An Atlantis dC18 5µm, 4.6x250 mm column (Waters Corporation, Milford, MA, USA) was used and column conditions were as follows: isocratic gradient of 75:25 water:acetonitrile for 0-3 min; linear gradient from 75:25 water:acetonitrile to 25:75 water:acetonitrile from 3-11 min; linear gradient from 25:75 water:acetonitrile to 75:25 water:acetonitrile from 11-16 min; isocratic gradient of 75:25 water:acetonitrile for 16-20 min. Flow rate was 1 ml/min; column temperature was maintained at 30°C. The corresponding fractions were collected according to the following retention times (in min): cyanuric acid 6.6; DDA 7.7; DIA 8.4; DEA 9.9; hydroxyatrazine 11.5; and atrazine 12.6. Fractions were then analyzed for radioactivity on a Tri-Carb 2900TR Liquid Scintillation Analyzer (Packard BioScience Company, Meriden, CT, USA). Non-radioactive analytical standards were used to determine retention times of the compounds on the HPLC and the beta-ram. The following non-radioactive standards were obtained: atrazine, DEA, DIA, and DDA from CIBA-GEIGY (Syngenta) (Greensboro, North Carolina, USA); hydroxyatrazine from Sigma-Aldrich (St. Louis, MO, USA); and cyanuric acid from Fluka (St. Louis, MO, USA).

2.3 Recovery efficiency

Recovery efficiencies for atrazine from plant for both studies was determined to be 97.5%. Recovery efficiencies for DEA, DIA, and DDA in plant material were determined to be 96%, 84%, and 90% respectively. Efficiencies for atrazine, DEA, and DIA recovery in soil were determined to be 91%, 85%, and 87% respectively. No further recovery efficiencies were

183 performed as it was assumed that based on the previously determined recovery percentages that
184 the compounds were related enough to produce similar results in the extraction procedures.
185 *2.3 Statistical Analysis*
186 As a result of incomplete data, no statistical calculations were performed for the
187 preliminary fate study. For the radiolabel study, SAS (SAS Institute Inc., Cary, NC, USA) was
188 used to perform an analysis of variance test on the concentrations of atrazine and its metabolites.
189 Comparisons were made between days for all compounds at $p = 0.05$.
190 **3. Results**
191 *3.1 Preliminary Fate Study*
192 *3.1.1 Atrazine and metabolite residues in soil*
193 Levels of atrazine and its metabolites were assessed over the course of the 21-day
194 experiment in soil (Figure 2). Extraction of soil on Day 0, after the first simulated runoff event,
195 yielded an atrazine concentration of 3.85 ± 0.21 μg/g (ppm) indicating that atrazine may not have
196 been evenly distributed throughout the soil. Extraction of soil from Day 1 yielded an atrazine
197 concentration of 15.85 ± 0.06 ppm, signifying that atrazine had reached uniformity throughout
198 the soil after 24 hours. Soil concentrations after the second and third applications were recorded
199 to be 23.5 ± 0.22 ppm and 38.9 ± 0.19 ppm on Days 4 and 7, respectively. After the final
200 simulated runoff event, levels of atrazine decreased to 13.57 ± 4.04 ppm by Day 11 and were not
201 detected in the soil by the final day of the experiment. DEA was detected in very low amounts on
202 Day 5 (0.80 ± 0.41 ppm). On Days 8 and 11, the concentration of DEA reached 1.43 ± 0.13 ppm
203 and 0.99 ± 0.16 ppm, respectively. DDA was detected only once in a single extraction (of 6 total)
204 on Day 14 (5.64 ± 5.64 ppm). The concentrations of DIA remained below the detection limit for
205 the entire experiment.

3.1.2 Atrazine and metabolite residues in leaf material

Concentrations of atrazine and its metabolites in above-ground switchgrass biomass were also assessed over the entirety of the study (Figure 3). Uptake of atrazine into above-ground biomass was observed beginning on Day 2 with the concentration reaching 4.43 ± 1.67 ppm. The concentration rose steadily to 7.91 ± 5.81 ppm by Day 4, but was undetectable throughout the remainder of the study by Day 8. The concentration of DEA reached 0.18 ± 0.18 ppm in the biomass on Day 4. Measureable levels of DEA were observed on Days 7 and 14. DIA was detected once on Day 14 (0.13 ± 0.13 ppm). DDA was found to be present on Days 7 and 14, 0.77 ± 0.25 ppm and 1.48 ± 0.50 ppm, respectively.

3.2 Radiolabel Study

One day after treatment, atrazine accounted for approximately 42% of the radioactivity recovered in the above-ground switchgrass biomass. DEA and DIA accounted for 28% and 20% of the radioactivity recovered, respectively. No other metabolites were observed in significant amounts. By Day 3, four metabolites were detected; DIA (36% of recovered radioactivity), DEA (25%), cyanuric acid (8%), and DDA (7%). Atrazine accounted for 20% of the recovered radioactivity. By Day 7, DIA accounted from 43% of the recovered radioactivity, more than double the next most prevalent metabolite, DEA (20%). Cyanuric acid (13%), DDA (13%), and atrazine (8%) were also detected in significant amounts.

Table 1 lists the concentrations of atrazine and its metabolites over the course of the radiolabel study. Atrazine increased slightly from Day 1 to Day 2, but significantly declined afterwards (p-value = 0.0015). Concentrations of DEA significantly increased from Day 1 to Day 3 (p-value <0.0001) before leveling off through the end of the study. DIA, DDA and cyanuric acid all increased significantly in concentration over the course of the study (see Table

2 for p-values). By Day 3, DIA was the compound detected in the highest concentrations in the above-ground biomass. Concentrations of hydroxyatrazine remained low throughout the entire study.

4. Discussion

4.1 Atrazine residues in soil

Typically, the concentration of atrazine found in the soil in the preliminary fate study rose to a level similar to the amount of atrazine applied via a simulated runoff event. This was not the case on Day 0; just hours after application only 24 % of the applied amount of atrazine was observed. It is possible that the atrazine applied was not given ample time to distribute evenly throughout the soil before sample collection because by 24 hours after each of the three simulated runoff events, the recorded levels of atrazine in the soil were consistent with the amount applied. Atrazine levels in the soil fell approximately 4.6 ppm between Days 1 and 2. One possible reason for this sharp decline can be attributed to plant uptake as the concentration of atrazine found in above-ground biomass on Day 2 was recorded at 4.43 ± 1.67 ppm. After the third simulated runoff event, atrazine levels peaked at 38.9 ± 0.2 ppm on Day 7. By Day 11 however, atrazine fell to a concentration of 13.6 ± 4.0 ppm. This decline in atrazine in the soil is probably the result of metabolism of atrazine by soil microbes as uptake of atrazine by switchgrass was limited after the third runoff application.

4.2 Metabolite residues in soil

Levels of DEA in soil began to appear as soon as Day 5 at a concentration of 0.80 ± 0.41 ppm and increased to 1.5 ± 0.8 ppm on Day 7. Levels of DEA were measurable on Days 8 and 11, peaking at 1.4 ± 0.1 ppm on Day 8 before declining for the remainder of the preliminary fate study. DDA was present once at a concentration of 5.6 ± 5.6 ppm. The metabolite DIA was not

252 detected in the soil over the course of the preliminary fate study possibly because there seems to
253 be a preferential removal of ATR's ethyl side chains in soils as opposed to removal of the
254 isopropyl group, causing deethylation reactions to proceed at two to three times the speed of the
255 deisopropylation reactions (Mills *et al*. 1994). Thus, production of DIA may be slow, but its
256 conversion to DDA could be much quicker (Mills *et al*. 1994). That is consistent with the data
257 we collected on DEA, DIA, and DDA. Finally, it should be noted that the presence of
258 metabolites of atrazine in the soil may be the result of plant degradation, microbial degradation,
259 or an interaction of plant and microbial degradative processes in the rhizosphere (Arthur *et al.*
260 2005). No attempt was made to attribute atrazine breakdown to plant or microbial degradation as
261 it was not within the focus of this study.

262 *4.3 Atrazine residues in leaf material*

263 Over the course of the preliminary fate study, switchgrass was shown to be capable of
264 taking up atrazine. A number of experiments have shown that various other plants are able to
265 take up atrazine including pea plants, corn, soybeans, cotton, and poplar trees (Burken and
266 Schnoor 1997, Davis *et al*. 1965, Shimabukuro 1967, Shimabukuro *et al*. 1966). Forty-eight
267 hours after the first simulated runoff event, atrazine could be found in the above-ground biomass
268 at a concentration of 4.43 ± 1.68 ppm. Using radiolabeled atrazine allowed more sensitive
269 detection of atrazine, in the parts per billion range (ppb), and thus atrazine was observed to be
270 present in the biomass as soon as one day following treatment with a simulated runoff. Levels of
271 atrazine in the biomass were undetectable by Day 8 and through the end of the preliminary fate
272 study. Even after the third simulated runoff event, levels of atrazine in the biomass continued to
273 decline. The fact that there was an ample amount of atrazine available in the system that could
274 potentially be taken up by switchgrass suggests that there may be a threshold on the amount of

contaminant that switchgrass is able to take up. This hypothesis of a threshold is also supported by the radiolabeled study which showed that the total concentrations of atrazine and its metabolites in the above-ground biomass increased from Day 1 to Day 3, but remained fairly constant for the remainder of the study. This kind of threshold has been observed previously in the roots of *Typha domingensis* which were shown to take up similar amounts of atrazine when exposed to concentrations of both 17 and 30 mg/L (Cejudo-Espinosa *et al*. 2009).

4.4 Metabolite residues in plant material

Concentrations of DEA found in the above-ground biomass during the preliminary fate study were recorded at measurable levels on Days 4, 7, and 14. Though DIA was only detected once over the course of the preliminary fate study at Day 14, using the more sensitive radiolabeled atrazine showed that DIA was also present one day after treatment. It was the most prevalent metabolite and increased in concentration over the course of the radiolabel study. Presence of DDA appeared in the biomass on Days 7 and 14 of the preliminary fate study. Again, use of the radiolabel allowed detection of DDA earlier than in the preliminary fate study, with measurable amounts first being detected on Day 3 and increasing over the remainder of the study.

This study focused primarily on the parent compound atrazine and its chlorinated metabolites, DEA, DIA, and DDA. While less toxic than the parent atrazine, DEA and DIA have been shown to have some toxic effects on green algae and aquatic plants (Stratton 1984, Jones and Winchell 1984). In addition, these metabolites of atrazine are of regulatory interest as they are included in the 90-day rolling average level of concern for atrazine in raw water (USEPA 2012). Hydroxylated metabolites were not studied in the preliminary fate study as they did not produce acceptable chromatographs in the GC system ustilized. Use of the radiolabel allowed

298 tracking of hydroxyatrazine in the radiolabel study which was not possible with gas
299 chromatography. Only very low concentrations of hydroxyatrazine were detected at any time
300 point in the study (Table 2). This is in contrast to earlier reports which showed that
301 hydroxyatrazine was detected in switchgrass tissues (Henderson *et al*. 2007, Lin *et al*. 2008). In
302 Henderson *et al.* (2007) the authors detected hydroxyatrazine in leaf biomass; however, they
303 used a mixture of switchgrass, yellow indiangrass, and big bluestem and extracted all the grass
304 tissue together. Thus, the presence of hydroxyatrazine in that study cannot be definitively
305 attributed to degradation by switchgrass. Lin *et al.* (2008) detected the presence of
306 hydroxyatrazine in switchgrass leaf biomass; however, they could not determine if the presence
307 of hydroxyatrazine was due to degradation by switchgrass or uptake from the soil. Therefore, it
308 is probable that the microbes present in the soil and the soil itself used in the current study may
309 not have transformed atrazine to hydroxyatrazine.

310 Lack of an optimized extraction method may explain the low amounts of hydroxyatrazine
311 recovered. To explore this, a second extraction technique modified from Lin *et al.* (2008) was
312 utilized on one sample from each column collected on Days 3 and 7. Recovery of
313 hydroxyatrazine with this method was approximately 77%. Using this adapted method resulted
314 in significant increases of the amounts of the polar metabolites cyanuric acid and DDA recovered
315 compared to the amounts recovered with the original method as well as significant decreases in
316 the amounts of atrazine and its nonpolar metabolites DEA and DIA recovered. The amount of
317 hydroxyatrazine recovered using the adapted method was still low. This provides more evidence
318 that switchgrass does not produce hydroxyatrazine as a metabolite.

319 The [^{14}C]atrazine also allowed the previously undetectable cyanuric acid to be detected,
320 as the ring structure containing the radiolabel, was still intact through much of the degradation

process. It is interesting to observe the presence of cyanuric acid, while not observing the presence of hydroxyatrazine, since both metabolites are de-chlorinated and hydroxylated (Figure 1). One potential explanation for this is that the enzymes present in the plant that catalyze the de-chlorination reaction may be unable to hydroxylate atrazine due to the presence of the ethyl and/or isopropyl groups and thus, the de-chlorination reaction could only occur after the removal of these groups.

4.5 Conclusions

The data presented here indicate that under simulated phytoremediation conditions containing switchgrass, atrazine is degraded into several metabolites and is below the limit of gas chromatography detection after 21 days. It is important to note that none of the chlorinated metabolites could be detected by the end of the preliminary fate study, suggesting that they had been degraded beyond the transformation compounds of interest. While it is possible that trace amounts of these compounds remain, they would be below the EPA's established tolerance of 4 ug/g for range grasses and thus, they should be of little regulatory concern (USEPA 2003). Using a radiotracer, the metabolites DEA and DIA were detected in above-ground biomass one day following a simulated runoff. DEA, DIA, DDA, and cyanuric acid were all determined to be present in significant amounts at various points throughout the study. Hydroxyatrazine was observed only at very low concentrations.

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