

# REDUCING ATRAZINE CONCENTRATIONS IN AGRICULTURAL RUNOFF THROUGH THE USE OF A CONSTRUCTED WETLAND

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*Abstract – Herbicides make up a large percentage of the synthetic pesticides used to enhance the agricultural productivity, and atrazine is one of the most commonly used herbicide in the United States. Once being applied either as a pre- or post-emergent herbicide, atrazine can be leached from the soil by dissolving in irrigation or rainwater. Hence, atrazine can contaminate natural waters such as drinking water, aquifers, and shallow groundwater beneath agricultural areas. In addition to its impact on fish, atrazine has potential short term and long term health effects in humans. The major objective of this study was to determine the effectiveness of a constructed wetland in reducing the level of atrazine in agricultural runoff. Water samples were collected after rain events over a one-year period from a wetland constructed to collect 85% of the pasture and cultivated field runoff from a large agricultural research station. The samples were prepared by solid phase extraction, and the concentration of atrazine was determined by HPLC. The results showed that the changes in the concentration of atrazine in the runoff generally paralleled the changes in the amount of rainfall that occurred just prior to sampling, indicating that rainfall leached the herbicide from the soil. Atrazine was detected in all samples on dates at the wetlands, suggesting that rainwater leached the atrazine in high amounts into the surface water; however, the constructed wetland was effective in reducing the atrazine concentration by a significant 52% before it reached the nearest public water.*

**Key Words:** wetland, atrazine, pesticide, herbicide, HPLC, watershed

## 1. INTRODUCTION

The use of synthetic organic pesticides has steadily increased worldwide since their commercial introduction following World War II, and of all the pesticides, the increased use of herbicides has been the most striking in the developed world [1]. Herbicides make up a large percentage of the synthetic pesticides used to enhance the agricultural productivity, and atrazine (1-Chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine) is one of the most commonly used herbicides in the United States [2]. When applied onto or incorporated into the soil, atrazine like many other herbicides, reaches the soil profile dissolved in irrigation or rainwater. It is absorbed through the roots by all plants, but it is not an effective herbicide in plants such as corn because it is broken down before it exerts its toxic affects [3]. In grasses and broad-leafed weeds that cannot break down atrazine, the chemical acts as a photosystem II inhibitor in the shoots and leaves, thereby killing the plant [4]. Hence, it is one of the most frequently applied herbicides in the agricultural sector for selective control of broad leaf and grassy weeds in crops like corn and sorghum [5]. About 85% of the 60–80 million pounds of atrazine applied annually in the United States is used on corn, with most of the rest being used on sugarcane and sorghum crops [6]. Atrazine can be applied pre-plant, pre-emergence, or post-emergence, making it a very flexible herbicide, but pre-plant and pre-emergent uses are the most popular [7].

Natural waters can be contaminated with various pesticides or their transformation products due to the fact they are directly applied to the soil and then transported into ground water and surface water, and atrazine is no exception. It can

be carried into water resources where it can potentially contaminate drinking water, aquifers, and shallow groundwater beneath agricultural areas [8]. Water quality monitoring studies find atrazine contamination 10 to 20 times more frequently than the next most detected pesticide [9]. The highest levels of atrazine in surface water occur in the spring and summer months following herbicide application [10]. The average concentration of dissolved atrazine for samples collected in June and September 1996 from Kansas watersheds was 0.20 µg/L, but fluctuated to as high as 7000µg/L in summer time and during rainfall events [11]. One reason that atrazine is an environmental concern is its low biodegradability [12]. It degrades very slowly once it enters the water column [13], and its half-life in reservoirs may be as much as 1 to 2 years [14]. Atrazine is also highly persistent in soil. The average half-life of atrazine in soil ranges from 13 to 261 days [15]; in river water more than 100 days [16]; in seawater around 10 days [17]; and nearly 660 days in areas of anaerobic degradation [18]. Atrazine concentrations of up to 108 µg/L have been reported in the rivers of North America [19].

Alterations in the chemical composition of natural aquatic environments can affect freshwater fauna, particularly fish. Atrazine has been shown to reduce plasma testosterone, olfactory sensitivity and salinity tolerance in mature male Atlantic salmon [20]. After washing from the field into streams and rivers with rainfall, atrazine can kill algae and other beneficial aquatic plants that provide food, shelter, and oxygen for aquatic animals [21]. Most human exposure to atrazine is from the consumption of contaminated ground

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water. Atrazine has potential short term and long term health effects. Short term potential health effects of ingestion of atrazine include heart, lung and kidney congestion, as well as low blood pressure whereas the potential long exposure at low levels include weight loss, retinal degradation, cardiovascular damage, and, potentially even cancer [22, 23]. Atrazine may also be an endocrine disrupter in humans [24], and there are reports on atrazine's adverse neuroendocrine and reproductive affects and the disruption of reproductive hormones including inhibition of luteinizing hormone (LH) release in laboratory animals [25]. Also, in toxicological studies with lab animals, exposure to chlorinated triazines, such as atrazine and its metabolites, has been shown to cause altered estrous cycles, delayed puberty, pregnancy loss, prostate inflammation, hermaphroditism and gonadal dysgenesis [26]. Studies on rats have shown increased occurrence of mammary gland tumors and endocrine perturbations after prolonged exposure to atrazine [27]. Likewise, in humans, exposure to atrazine has been associated with intrauterine growth retardation (IUGR) [28], small-for-gestational-age fetuses (SGA) [29], spontaneous abortion [30], and reduced semen quality [31]. Although several countries gave up the use of atrazine because of its toxicity, it is still one of the most popular herbicides in many countries including the United States [32].

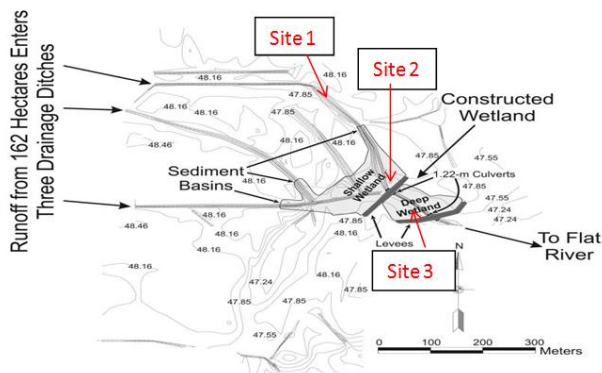
One of the major factors that influences pesticide runoff is the time elapsed between pesticide application and a rainfall event [33]. The wetter the soil surface at the time atrazine is applied, the sooner runoff begins during a rain and the greater the potential for atrazine runoff, but rainfall that soaks into the soil prior to runoff will move some of the atrazine below the mixing zone, reducing the amount of atrazine subject to runoff losses [10]. Rainfall varies with time and space. A study on pesticide movement into estuarine systems by Alegria and Shaw [34] have shown that in general, pesticides will be transported via runoff in the dissolved phase and that most transport occurs within a few weeks of application, depending on local precipitation. Heavy rainfall and full streams lead to the highest pulse concentrations of atrazine, indicating that it is a readily available constituent in the watershed that is being washed off in proportion to the amount of excess rainfall runoff [35]. The greater the distance from field to surface water, the less likely it is that significant amounts of atrazine will enter the body of water [36]. From 1992 to 1996, seventy-six pesticides and seven pesticide degradation products in 8,200 samples of ground and surface water were analyzed in Midwestern states. More than 95% of all samples collected from streams and rivers contained at least one pesticide, and atrazine and its degradation product de-ethylatrazine (DEA) were among the most frequently detected pesticides in agricultural areas [37]. There have been other reports of the incidental runoff of atrazine into streams adjacent to agricultural land, and this has created concern about the potential impact on water quality and aquatic life [7, 38].

Typically, wetlands have been shown to successfully ameliorate water contaminated with phosphorus, nitrogen, hydrocarbons, animal waste, and heavy metals, and constructed wetlands have been found to be an effective

option for on-site wastewater treatment when properly designed, installed, and maintained. Constructed wetlands are wetlands created from non-wetland sites for the purpose of treating wastewater [39]. Constructed wetlands consist of saturated substrates, emergent and submerged vegetation, invertebrates and vertebrates, aerobic and anaerobic microbial populations, and a water column [39]. The objective of this study was to determine the effectiveness of a constructed wetland in reducing the level of atrazine in agricultural runoff. The Louisiana State University Agricultural Center Red River Research Station provided an ideal location for this study. The Red River Research Station (RRRS) consists of 162 ha of agricultural land in the Red River Basin of northwest Louisiana [40]. At this research station, cotton, soybean and corn are the major crops, and the site is used for field testing of the effect of different pesticides and fertilizers, including the application of atrazine to corn and sorghum. Since 1998, the Red River Research Station has utilized a constructed wetlands located in the southeastern corner of the station to conduct research to identify practices that minimize the impact of agricultural production on the quality of runoff water [40]. The amount of rainfall can be significant and result in considerable leaching and deposition of sediments at the station. The effectiveness of a constructed wetland in improving the water quality of agriculture runoff increases with the time runoff remains in the wetland system before being released to a receiving body [41]. Approximately 85% of the runoff water from the RRRS flows through this constructed wetland before eventually draining into a nearby river. Previous studies at the RRRS have shown that a constructed wetland on the site improves the water quality from runoff from the nearby farmland [42; 43], but there have been few studies on the effectiveness of the constructed wetland on reducing the discharge of atrazine in a full-scale agricultural setting.

## **11. METHODS AND MATERIALS**

**Sampling Sites:** The RRRS consists of cultivated fields and pasture land. The cultivated fields are intersected by three drainage canals to control excessive rainfall. One of these canals also drains pasture land that houses a small cow/calf herd (less than 100 animals), and it was chosen to be the first sampling site (Figure 1). Discharge water from the cultivated acreage and most of the pasture land on the station flows through these canals to the southeastern corner where they enter the first of two man-made ponds as shown in Figure 1[42]. The drainage canals enter a shallow pond which then feeds into a larger, deeper pond. The deeper pond serves as a holding tank to contain the field runoff before entering the Flat River which is less than a third of a mile away. These two locations were the second (shallow pond) and third (deep pond) sampling sites (Figure 1). Samples were collected on the same day or a day after a heavy rainfall in the area. Data were collected from October, 2012 to November, 2013 depending upon rainfall and time availability. The actual rainfall at each specific site was not determined but data from Shreveport Regional Airport (SHV), Downtown Airport (DTA) and Barksdale Air Force (BAF) provided a measure of the general rainfall in the area. The RRRS is relatively close to BAF; therefore the rainfall data reported in this study is that recorded at BAF.



**Figure 1: Location of construction wetlands and the pathway of discharge to Flat River [42]**

**Sampling and Sample Preparation:** Collection of the samples was accomplished by means of an alpha water sampler unit made by Wildco, model 1130-G45. The sample was transferred from the alpha jar to a 1000 mL amber glass bottle and stored for 4°C for transfer to the lab [44], and the sample was prepared for analysis within 24 hours of collection [45]. In preparation for solid phase extraction (SPE), the samples were first passed through a series of filters to remove undesired debris and organisms from the samples [46]. After the desired clarity was achieved using a Buckner funnel with Whatman 47mm 541 hardened ashless filter paper, a Millipore 0.45 micron pore size 47mm diameter filter attached to a vacuum [47] served to remove fine-contaminant material from the sample.

**Chemicals:** High pressure liquid chromatography reagents purchased from Fisher Scientific (USA) consisted of HPLC grade methanol ( $\geq 99.9\%$  purity), which was used in the preparation of the SPE cartridges as well as the mobile phase of HPLC. Pure atrazine was obtained from Sigma-Aldrich (St. Louis, Missouri, USA), and a serial dilution of atrazine was prepared to establish the standard curve. Only two solvents, methanol and deionized (DI) water, were used throughout the SPE and HPLC process.

**Solid Phase Extraction (SPE):** Solid phase extraction (SPE) as used by Putnam [48] was used to extract the triazines from the water samples. The samples were filtered through LC-18 3mL Supelco Solid Phase Extraction tubes which were treated with a 2 mL methanol wash. Supelclean Environmental Cartridges produced by Supelco Analytical (USA) and custom semi-automated equipment built on-site [43] were used to concentrate the samples. The cartridge (Supelclean ENVI-18) had a 500 mg bed weight, operated at a volume of 3 mL, had a surface area of 490 m<sup>2</sup>/g, an average particle size of 51.7 microns, and an average pore diameter of 73 angstroms. The clean cartridges were conditioned in a two-step process for the preparation and analysis of samples. Throughout the conditioning process, a 15 mmHg vacuum was pulled through the cartridge to allow complete dispersal through the bed. The two step conditioning process consisted of the application of 5 mL of methanol followed by 5 mL of deionized water at a flow rate of 3 mL/min [49]. After 10 cartridges were conditioned, they were loaded into a custom

built semi-automatic vacuum manifold and processed simultaneously. Once attached to the manifold, a volume of 500 mL of the sample was passed through the ENVI-18 cartridge at a flow rate of 3 mL/min. Precise flow rate was controlled through the use of a gravity fed drip control method which delivered the water samples directly to the cartridge. A 20 mmHg vacuum was applied to the ENVI-18 cartridges as the samples ran through them. This allowed for the herbicides to attach to the cartridge while the other contaminants were pulled through with the eluent. The vacuum was monitored through the use of a REP vacuum gauge; precise control of the vacuum was controlled through the use of a bleed valve which allowed positive pressure into the manifold to ensure that each cartridge had the exact same vacuum. Once the entire 500 mL water sample was processed through the cartridge, the vacuum was allowed to run for an additional two hours to allow for proper drying of the cartridges [49]. Once dried, the ENVI-18 cartridges were removed from the vacuum manifold for elution with methanol. Elution of the cartridges was performed by passing a total of 8 mL of methanol dispensed in two steps of 4 mL each with a 5 minute delay [47]. The collection of the compounds eluted from the cartridges was accomplished through the use of an attachment to the vacuum manifold which allowed the operator to control the suction placed on the cartridge as the methanol was pulled through. Eight mL of elute were collected in a test tube for each sample. The 8mL of sample were then transferred to conical drying tubes. Nitrogen was run through the conical drying tubes, and about 95% of methanol was removed. In the final preparative step before analysis, the dried extracts were brought up to a final volume of 100 $\mu$ L with a HPLC grade methanol [50]. After resuspension of the concentrated samples, they were placed into 100 $\mu$ L HPLC auto sampler vials. At this point the compounds were stable and could be placed into the cold storage at -18°C until ready for HPLC analysis.

**Chromatographic and Analysis Conditions:** A Dionex HPLC Ultimate 3000 consisting of the pump, autosampler, column compartment, diode array detector and fluorescence detector was used in the study. The optimum mobile phase for atrazine was found to be methanol/DI water, 70/30 v/v, at a flow rate of 1.0 mL/min [51]. The sample volume injected into Dionex HPLC system was 20  $\mu$ L. The analytical column used for separation in the study was an Acclaim 120 C18, 4.6  $\times$  150 mm, 5  $\mu$ m (Dionex-3000). Detection was carried out at a wavelength of 220 nm [51].

**Peak Identification and Quantification and Data Analysis:** Chromeleon 7.1 software by the Dionex Corporation was used for the identification and integration of peaks. For the standard, a known concentration of atrazine was prepared by dissolving atrazine in methanol and preparing a series of ten dilutions from this initial standard. After running the calibration standards, the Chromeleon 7 software was able to automatically detect and determine the retention times as well as the concentration of each atrazine standard. The field samples were processed the same way, and one standard was also run with each set of field samples for the quantification and localization of the atrazine peak. Identification of peaks was not automated, but the retention time on the standard gave the operator a clear determination of the atrazine peaks. All data points are based on a mean of

the measurements taken from a minimum of three samples. All data were subjected to a one-way analysis of variance, and significance was determined at the 95% confidence limits.

### III. RESULTS AND DISCUSSION

Various studies dealing with the movement of pesticides in water have concentrated on measuring runoff from sites of application and transport to groundwater, streams, and estuaries [34]. A comparison of the rainfall amount and the concentration of atrazine in the drainage canal in the current study is shown in Figure 2. The changes in the concentration of atrazine in the runoff generally paralleled the changes in the amount of rainfall that occurred just prior to sampling, indicating that rainfall leached the herbicide used at the station and deposited it in the constructed wetlands via runoff. Atrazine is usually applied once a year to the soil surface and has a relatively long (6 month) half-life in the soil [14]. This half-life plus its high solubility results in the highest concentrations of atrazine occurring in runoff water soon after the first few rain events or irrigation events following application [52]. Hence, as a rule, the largest atrazine loads in streams are likely to occur over a relatively short time [53, 54]. This could explain why the peak in atrazine concentration occurred in the runoff water resulting from the heavy rainfall in April, 2013.

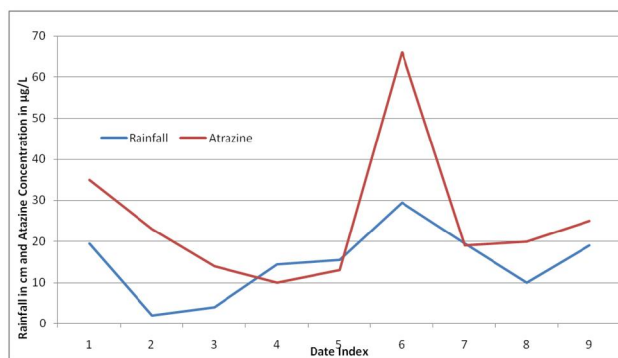


Figure 2: Comparison of atrazine concentration in the field canal and rainfall amount on or the day after the following dates: (1 = October, 14, 2012; 2=October 26, 2012; 3 = Dec 13, 2012; 4 = Jan. 9, 2013; 5 = Feb. 10, 2013; 6 = April 10, 2013; 7 = Sept. 20, 2013; 8 = Sept. 26, 2013; 9 = Nov. 22, 2013).

The atrazine concentrations in the samples collected at site 1 (field canal which drains runoff water into the shallow pond), site 2 (shallow pond) and site 3 (deep pond) are shown in Figure 3. Samples collected from the field drainage canal showed the highest concentrations of atrazine. The highest concentration observed in the field canal was 67.3 µg/L and occurred in April following the annual application to corn plots and significant rainfall. This is comparable to runoff water values reported by Wauchope [14], although values as high as 800 – 1400 µg/L have been reported following severe storm conditions [55, 56]. In the months with very little rainfall (January and February, 2013), the atrazine concentration remained somewhat static at around 10 µg/L throughout the wetland; however, in most of the samples collected when there was sufficient rainfall to produce runoff,

there were significant decreases in the atrazine concentrations when the water was held the shallow pond, and the levels decreased even more when the water reached the deep pond. Comparison of the average atrazine concentration detected in the field drainage canal and deep pond showed that the deep pond had 25.74% less atrazine than the field drainage canal. Comparison of the average atrazine concentration between the shallow pond and the deep pond showed the atrazine concentration in the deep pond to be an average of 26.24% less than the shallow pond. Overall, the average amount of atrazine entering to the wetlands was about 52% higher than the amount discharged to the Flat River.

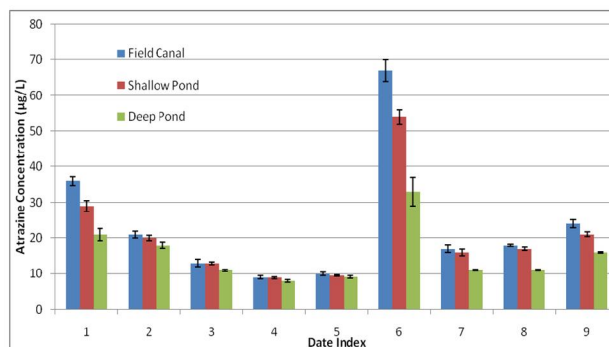


Figure 3: Comparison of atrazine concentrations ( $\mu\text{g/L} \pm \text{SE}$ ) in the field drainage canal, shallow pond and deep pond at RRRS on the following dates: (1 = October, 14, 2012; 2=October 26, 2012; 3 = Dec 13, 2012; 4 = Jan. 9, 2013; 5 = Feb. 10, 2013; 6 = April 10, 2013; 7 = Sept. 20, 2013; 8 = Sept. 26, 2013; 9 = Nov. 22, 2013).

Atrazine degradation in soil is mainly by photolysis and microbial processes, but it is relatively stable in the aquatic medium under environmental pH conditions [11]; however, in the current study, it is obvious that considerable degradation occurred in the constructed wetland. Atrazine degradation can result from both biological and non-biological processes in aquatic and soil environments [38]. The features of atrazine such as slow hydrolysis, low vapor pressure, moderate aqueous solubility, and its resistance to microbial degradation slow its degradation rate and enhance its potential for contaminating ground water [57]. Other studies [58] have shown that the atrazine levels in wetland sediments and plants were below the detectable level, suggesting that atrazine degradation does not occur in either of these wetland components. On the other hand, atrazine can be degraded in surface water by photolysis and microorganisms via *N*-dealkylation and hydrolysis of the chloro-substituent [9]. Studies under microcosm conditions by Runes *et al.* [59] demonstrated that bioaugmentation of a constructed wetland could enhance atrazine degradation when compared to a non-bioaugmented wetland. Thus, in the present study, it is believed that a significant portion of the constructed wetland's treatment of runoff is the result of transformation and detoxification of compounds such as atrazine by microorganisms residing in the water and on the rhizomes of native wetland plants. Dilution of atrazine in runoff may be significant once runoff exits the constructed wetland as all water during a rainfall event is not collected in the wetlands.

A few other studies have shown that constructed wetlands can effectively remediate atrazine levels in runoff water. In relative small (59 – 73 X 14 X 0.3 m) experimental constructed wetlands, Moore *et al.* showed that constructed wetlands could reduce atrazine concentrations after treatment of wetland cells with aqueous atrazine followed by a one-time simulated rainfall equivalent to three volumes within each wetland cell, provided the buffer travel distance within the wetland ranged from 100 – 280 meters [58]. The current study utilized a much larger constructed wetland (approximately 150 X 100 X 0.5 m for the shallow pond and 100 X 75 X 2.5 m for the deep pond) that received runoff containing leached atrazine from soil where the herbicide was normally applied at standard rates during typical agricultural activity at an experimental agricultural research facility. In addition, the total length of the wetland zone exceeded 300 meters. Runes *et al.* [59] utilized a microcosm approach to show that a constructed wetland augmented with soil containing microorganisms from an atrazine spill site was more efficient at remediating atrazine contaminated water than a control constructed wetland that had not been augmented. In the present study, the constructed wetland consisted only of the existing soil at the construction site and native wetland plants that were relocated to the wetlands area. In conclusion, it can be stated that under actual agricultural conditions, atrazine was detected in all samples on all dates at the wetlands, indicating that rainwater leached the atrazine in high amounts into the surface water; however, the constructed wetland was effective in removing significant amounts of atrazine before it reached the Flat River.

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