Journal of Hazardous Materials 284 (2015) 103–107

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Nucleophilic substitution as a mechanism of atrazine sequestration in soil

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HIGHLIGHTS

- Atrazine tends to form nonextractable bound residue in soil.
- Nucleophilic substitution is a pathway leading to atrazine sequestration in soil.
- Sulfur containing amino acids are likely to play an important role as nucleophiles during this process.

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 19 October 2014
Accepted 3 November 2014
Available online 7 November 2014

Keywords:
Atrazine
Sequestration
Bound residue
Nucleophic substitution

ABSTRACT

Formation of nonextractable residue was widely observed as a sink of atrazine (ATZ) in soil. However, the mechanisms by which ATZ binds to soil organic matter remain unclear. In this study, we demonstrated that neucleophilic substitution could serve an important pathway causing ATZ sequestration. The carbon bonded to the chlorine in ATZ molecule is partially positively charged due to the strong electronegativity of chlorine and is susceptible to the attack of nucleophiles such as aniline. Since aromatic amines are relatively rare in natural soils, amino acids/peptides were hypothesized to act as the main nucleophiles in real environment. However, substantially ATZ transformation was only observed in the presence of those species containing thiol functionality. Thus, we speculated that it was the thiol group in amino acids/peptides acting as the nucleophile. Nitrogen in amino acids was in fact not an active nucleophile toward ATZ. In addition to the sulfur-containing amino acids, other thiol compounds, and sulfide were also proved to be reactive to ATZ. Thus, the sequestration potential of ATZ probably correlates to the availability of thiol compounds in soil.

1. Introduction

Atrazine (1-chloro-3-ethylamino-5-isopropylamino-2,4,6-triazine, ATZ) is the most commonly applied herbicide to control broadleaf weeds and grasses. It was estimated that over 50 million pounds of active ATZ ingredients are applied annually in United States to corn, soybeans, sorghum, and other crops [1]. Once applied to the soil, ATZ has great chance to enter surface and ground waters through runoff or infiltration [2,3], because it is water soluble and relative conservative in the environment with low volatility, weak sediment partitioning, and relatively slow degradation rates (i.e., a half-life of months to years) [2]. As a result, ATZ is the most commonly detected herbicide in ground and surface waters [1,4]. The U.S. EPA has set the drinking water maximum contaminant level (MCL) at 3 ppb, but ATZ concentrations often rise far above this level, especially after herbicide application and during runoff [5,6].

ATZ is a contaminant of environmental concern because its presence has been associated with causing imbalances in hormone levels in animals, possibly disrupting reproductive, and developmental processes [7–9]. Short term ATZ exposure above the drinking water MCL can potentially cause heart, lung, and kidney congestion, low blood pressure, muscle spasms, weight loss, and damage to the adrenal glands. Long term exposure to ATZ above the drinking water MCL has been linked to weight loss, cardiovascular damage, retinal, muscle degeneration, and cancer [10]. The high solubility and mobility of ATZ in combination with its relative resistance to degradation and significant health impacts makes ATZ contamination a serious threat to the environmental and human health [11], particularly in rural communities where ATZ is frequently used in agricultural activities but effective water treatment measures are not in place.

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http://dx.doi.org/10.1016/j.jhazmat.2014.11.001
0304-3894/

A thorough understanding of ATZ transformation in environment is critical for scientifically assessing the environmental risks associated with its application and for devising effective management and remediation means to mitigate its negative environmental and human health impacts. Extensive studies have been done in this topic and useful information obtained. ATZ undergoes both biotic and abiotic transformation in soil through two primary types of pathways: degradation and sequestration. While information regarding ATZ degradation is abundantly available [12–16], that relating to its sequestration is scarce. Evidences from experiments using ^{14}C-atrazine indicted that ATZ sequestration indeed occurred in soil resulting in the formation of ATZ bound residue with soil organic matter (SOM). The extent of such bound residue formation however varied greatly from case to case, which seemed to depend on factors that had yet to be understood. Barriuso and Koskinen found that nonextractable residues were formed immediately after atrazine application to unsterilized agricultural soil, and increased with the herbicide residence time in soil [17]. Jablonowski et al. tested a lysimeter soil containing long-term aged ^{14}C-ATZ for over twenty years and found 8.8% of the original ^{14}C activity remained in the top soil, most of which was bound to soil organic matter and could not be extracted [18]. Lesan and Bhandari concluded that longer atrazine-soil contact times resulted in enhanced nonextractable residue formation, and over an 84-day contact period, 35–50% of the pre-loaded ^{14}C-ATZ was found ending with residues in the organic components of soil [19]. All the above studies show distinctly that sequestration of ATZ and/or their metabolites occurs in soil, but mechanistic understanding about ATZ sequestration is still limited. In this research we demonstrated that neucleophilic substitution was a potential pathway leading to ATZ sequestration in environment, and certain sulfur containing amino acids/peptides probably played an important role as nucleophiles during this process.

2. Experimental

2.1. Chemicals and material

ATZ was purchased from Sigma–Aldrich (St. Louis, MO). Phenol, gallic acid, catechol, resorcinol, quinol, salicylic acid, veratry alcohol, 4-methoxylphenol, aniline, glycine, cysteine, glutathione, methionine, mercaptoethanol, and Na_2S were all purchased from Aladdin (Shanghai, China). HPLC grade methanol and acetone were also from Sigma–Aldrich. Other chemical were reagent grade or better. All solutions were freshly prepared in deionized water.

2.2. Reaction setup

Reactions between ATZ and SOM were first explored in solutions in a series of glass vials as batch reactors. Each reactor contained 50 mL solution of 0.05 mM ATZ and one of the humic constituents (phenolic/anilinic compounds or amino acids listed in the previous paragraph) at concentrations from 0 to 50 mM. The pH of the solution was buffered at 5.8 using 1.0 mM phosphate. The reactors were kept in dark in an incubator maintained at 20 °C. After predetermined incubation time, 1.0 mL sample was taken from each of the vials and analyzed using a Hitachi L-2000HPLC equipped with a photo-diode array (PDA) detector. A C18 reverse phase column (Hitachi LaChrom, 5 μM × 250 mm × 4.6 mm) was used for separation. An isocratic elution consisting of 70% methanol and 30% water at a flow-rate of 1.0 mL/min was used as mobile phase. Controls with only ATZ were also prepared. Three replicate experiments were performed for each reaction condition.

Reactions of ATZ was further explored in soil samples spiked with humic constituents and amino acids that were determined to be reactive to ATZ in previous experiments. The soil of yellow brown earth was collected from Xiamafang Park in Nanjing, China. The soil was dried, grinded, and sieved through 20-mesh screen before use. The pH of the soil was 5.73. Each reaction sample contained 5.0 g dry soil and sterilized before mixing with appropriate amount of ATZ dissolved in acetone to achieve a concentration of 10 mg/kg. After acetone was evaporated, 2.0 mL solution of 50 mM humic constituent or amino acid was spiked. The molar ratio of ATZ/humic constituent was approximately 1/440. After the soil samples was incubated in 20 °C in dark for 6 day, the residue ATZ was extracted with 10 mL acetone twice facilitated with sonication. The extracts were combined and concentrated to 1 mL using a gentle stream of nitrogen gas. ATZ in the sample was quantified as described above. Control samples spiked with only ATZ were prepared and treated using identical procedure. Three replicates were performed for each reaction condition.

2.3. Products characterization

To characterize the reaction products, selected samples with substantial ATZ removal were analyzed using an Agilent G6410B Triple Quad Mass spectrometer with an electron spray ionization source. The ionization source was operated in positive mode (ESI+). Nitrogen was used as desolvation gas and maintained at flow rate of 10 L/min. The desolvation temperature was set at 350 °C. The mass analyzer was first run at scanning mode from m/z 100 to 1000. Secondary MS of the suspected reaction products were obtained subsequently. Fragmentor and collision energy were experimentally optimized.

3. Results and discussion

3.1. Removal of ATZ in the presence of humic constituents

The complexity of SOM renders difficulty in the elucidation of xenobiotic–SOM interactions. A variety of substituted phenolic and anilinic compounds, so called humic constituents that mimic the structure of SOM were incubated with ATZ in an attempt to identify the specific functionalities that form covalent bonds with ATZ. It was revealed in Fig. 1 that ATZ was removed to different extent in the presence of different phenolic compounds. No reduction was seen in samples incubated with phenol or salicylic acid after 5 months. Appreciable attenuation was found in samples with gallic acid, veratry alcohol, catechol, resorcinol, quinol, and 4-methoxylphenol. Nonetheless, the removal in the presence of aniline was most pronounced and precipitants were observed. The removal increased consistently over time and exceeded 30% after

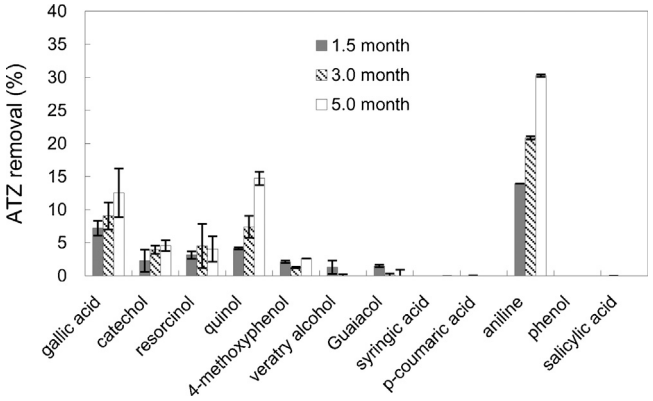

Fig. 1. Removal of ATZ in the presence of humic constituents.
(Conditions: $[ATZ]_0$ = 0.05 mM, initial concentration of the humic constituent was 0.50 mM, pH 5.8).