CHAPTER 29

ENHANCED ADSORPTION OF ATRAZINE IN DIFFERENT SOILS IN THE PRESENCE OF FUNGAL LACCASE

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Abstract: Adsorption–desorption behavior of atrazine was studied in three soils belonging to different soil geographical zones. Experimental investigations focused on the effect of laccase addition on adsorption and desorption of target chemical when present in solution. Addition of laccase resulted in a dramatic increase in adsorption of the atrazine. Desorption was little or negligible. Hysteresis, represented by hysteresis indices, was significantly enhanced upon laccase addition. Increases in Freundlich K_F values upon laccase addition were attributed to the covalent bonding of atrazine to soil organic matter by oxidative coupling mechanism.

Keywords: atrazine; adsorption; desorption; soil; laccase; oxidative coupling

Introduction

Agricultural chemicals, while often benefiting agricultural productivity, can have detrimental environmental effects when applied improperly. Herbicides, such as chlorotriazines in general or atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) in particular, are most frequently detected in both terrestrial and aquatic ecosystems due to their relatively high mobility and persistence in the environment (Huber, 1993; Solomon et al., 1996; Graymore et al., 2001). Atrazine is one of the most widely used pesticides for the control of both grasses and broadleaf weeds in many crops and in nonagricultural

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situations such as on railways, highways, and industrial sites. The concentration of atrazine can exceed the general quality standard for surface water, with maximum reported values of 9–25 mg/L in Europe (Croll, 1991; Legrand et al., 1991), 87–100 mg/L in North America (Steinheimer, 1993; Graymore et al., 2001), and up to 150 mg/L in Australia (Graymore et al., 2001).

Atrazine has several different fates in the environments including adsorption–desorption, migration, decomposition, and so on. (Gao et al., 1998; Mersie et al., 1998). However, adsorption–desorption processes are often supposed to control the other fates including both translocation into plants, movement in soil profiles or aqueous systems, abiotic or biological decay, and so on.

To describe the overall sorption characteristics of a particular soil at equilibrium with a range of contaminant concentrations adsorption isotherms, such as the Freundlich isotherm, are often used. The Freundlich model for atrazine adsorption can be described by the equation:

$$\text{Atrazine}_{\text{sorbed}} = K_{\text{F}}[\text{Atrazine}]^{n_{\text{F}}}, \tag{1}$$

where $\text{Atrazine}_{\text{sorbed}}$ and [Atrazine] represent the solid- and aqueous-phase equilibrium concentrations of the target chemical, respectively; the Freundlich constant K_F is a measure of the sorption capacity of the sorbent; and the Freundlich n_F measures sorption linearity, which is related to the heterogeneity of sorption site energy.

There are several physical–chemical characteristics of the soil affecting adsorption and desorption of atrazine such as organic carbon (OC) contents, acidity, surface area, electric potential of the clay surface, and others (Lee et al., 1990), but organic matter are known to be the leading one among them (Seta and Karathanasis, 1997). This was numerously shown in the literature as positive correlation between atrazine Freundlich constant K_F or distribution coefficient Kd (equal to K_F when n_F=1) and content of OC (Mersie and Seybold, 1996; Moreau and Mouvet, 1997; Moorman et al., 2001; Ben-Hur et al., 2003). The Kd coefficient can be normalized to the fractional OC content of soil to give the relatively invariable partitioning coefficient, K_{OC} (Karickhoff, 1984), widely used for predictions of pesticides retention by soils in environmental and agricultural practice. In the database compiled by Wauchope et al. (2002), an average K_{OC} of 100 L/kg and a range of K_{OC} values from 38 to 174 L/kg are listed for atrazine; the upper limit for this value found in literature is 650 L/kg (Ben-Hur et al., 2003).

While adsorption is an important mechanism to examine, desorption is equally significant since it is directly related to herbicide runoff and leaching that leads to surface water and aquifer contamination, respectively. Barriuso

et al. (1994) found that atrazine adsorption was generally reversible for smectites, suggesting weak van der Waals forces or hydrogen bonds between the atrazine molecules and the clay surfaces. For soil samples, particularly those high in OC, however, adsorption is typically less reversible. Many researchers have found that atrazine exhibits hysteresis effects, that is, less herbicide desorbs than is predicted by adsorption isotherms. Hysteresis reflects the portion of contaminant that is very strongly or irreversibly bound to the soil or OC, and has been recently described by the hysteresis index H (Celis et al., 1997):

$$H = n_{\mathrm{Fa}} / n_{\mathrm{Fd}}, \tag{2}$$

where n_{Fa} and n_{Fd} are indexes of nonlinearity of the adsorption and desorption isotherms, respectively.

Although adsorption–desorption processes between atrazine and soil components have been well studied (Lesan and Bhandari, 2000; Ben-Hur et al., 2003; Magezan et al., 2003), chemical reactions between these pollutants and soil/sediment matrices have not been investigated in great detail. There is increasing evidence, however, that chemical interactions between organic pollutants and soil components, specifically reactions catalyzed by transition metal oxides or extracellular soil enzymes (so-called “oxidative coupling” or “oxidative polymerization reactions”), can significantly affect the fate of contaminants in soils and sediments, and potentially alter the associated health risks from the chemicals. The latter was numerously demonstrated for organic pollutants of phenol and amine structure (Voudrais and Reinhard, 1986; Wang et al., 1986; Nannipieri and Bollag, 1991; Bollag, 1992; Gianfreda and Bollag, 1994). It was elucidated, for example, that in the case of phenols the contaminants could be fixed or “trapped” within soil matrices as a result of enzymatic processes that imitate humus formation (ibid.).

Soils contain a large background concentration of extracellular enzymes that catalyze degradation and biosynthesis reactions in the soil environment. These organic catalysts are often protected against natural degradation by their attachment to soil constituents. Several soil enzymes including peroxidases, laccases, and polyphenol oxidases are capable of catalyzing chemical reactions that result in the polymerization of hydroxylated aromatic compounds (Sjobald et al., 1976; Suflita and Bollag, 1980; Sarkar et al., 1988). However, only single study on incorporation of atrazine by oxidative polymerization reactions into soils was found (Barriuso and Koskinen, 1996) though atrazine is known to belong to herbicides readily forming nonextractable bound residues, which are closely related to OC content in soil (Calderbank, 1989; Loiseau and Barriuso, 2002; Munier-Lamy et al., 2002).

Among enzymes catalyzing oxidative coupling, laccase seems to be the most important both from designing engineered remediation systems and controlling bound residues formation in nature points of view. This supposition is governed by the fact that this enzyme is only keeping relatively constant activity in soils all over the year (Criquet et al., 2000; Mayer and Staples, 2002). Besides, laccases possess wide substrate specificity catalyzing the oxidation of a variety of aromatic hydrogen donors with the concomitant reduction of oxygen to water. Moreover, laccases have been reported to oxidize many recalcitrant substances, such as chlorophenols, PAHs, lignin-related structures, organophosphorous compounds, nonphenolic lignin model compounds, phenols, and aromatic dyes in the presence of appropriate redox mediator (Mayer and Staples, 2002). The main feature of laccase action is its ability to produce a free radical from a suitable substrate. The mechanism of xenobiotics bioremediation using laccase is based on this reaction.

The aim of the study was to evaluate influence of fungal laccase on atrazine adsorption–desorption behavior in different soils.

Experimental Part

SOILS

Three soil surface samples (0–5 cm) used in the adsorption–desorption experiments were collected from forest or agricultural sites of different soil geographical zones and included sod-podzolic soil (Moscow region, forest site; related to Spodosols), gray forest soil (Tula region, agricultural site; related to Alfisols), and chernozem (Kursk region, agricultural site; related to Mollisols). Soil samples were air-dried, passed through a 1 mm sieve, and stored at room temperature prior to testing. Their physical–chemical properties are summarized in Table 1.

Based on the soil texture analysis, sod-podzolic and gray forest soil were classified as silt loam, and chernozem was classified as silt clay loam.

ASSAY OF LACCASE ACTIVITY IN SOILS

To perform assay of laccase activity, soils under study samples were extracted with 140 mM sodium pyrophosphate (pH 7.1) at soil to solution ratio 1:10 (w/w) at 25°C for 24 h in accordance with Bonmati et al. (1998) followed by enzyme activity assay using PerkinElmer spectrophotometer (USA) in temperature-controlled 1-cm cuvette at 25°C.

Laccase activity was measured using syringaldazine (4-hydroxy-3, 5-dimethoxybenzaldehyde azine, Sigma, USA) as a substrate. The reaction mixture contained 3 mL of 0.1 M syringaldazine in 0.1 M acetate buffer (pH 4.5). The reaction was initiated by the addition of 0.05 mL of

TABLE 1. Physical and chemical properties of soils

			Exchangeable bases, mM eq./100 g			
Soil	pH	OC, %	Ca	Mg	K	Na
Sod-podzolic	4.7±0.1	3.77±0.02	3.1±0.5	1.2±0.5	0.27±0.03	nd[a]
Gray forest	6.7±0.1	2.01±0.02	11±2	9±2	0.49±0.03	nd
Chernozem	6.6±0.1	5.79±0.02	31±4	7±5	0.47±0.06	0.4±0.2

[a] Below detection limit

sodium pyrophosphate extract from soil and the increase in absorbance was monitored at 530 nm. One unit of enzymatic activity was defined as the amount of enzyme required to cause a change in absorbance of 0.1 per minute at 25°C.

CHEMICALS

Atrazine

Atrazine (99.97%) was purchased from Dr. Ehrenstorfer GmbH (Germany). Stock solution of 1 g/L was prepared in methanol (Sigma, USA) and stored in the dark at 4°C.

Laccase

For this study, laccase (EC 1.10.3.2) form the strain of basidiomycetes *Coriolus hirsutus* 075 (Wulf. Ex. Fr.) Quel. of the *Polyporaceae* family producing high-activity extracellular laccase was used. The strain from the Collection of the Komarov Botanical Institute, Russian Academy of Sciences (St. Petersburg) was kindly provided by Dr. V. Gavrilova. Extracellular laccase was isolated from the culture medium and purified in accordance with (Koroleva (Skorobogat'ko) et al., 1998). The control of laccase homogeneity was carried out with polyacrylamide gel (PAAG) electrophoresis under nondenaturing conditions as described in Westermeier (2001). The pH optima of the major isoenzyme determined using pyrocatechol as substrate was 4.5 (Koroleva (Skorobogat'ko) et al., 1998). Stock solution of 17.5 g/L was prepared in 50 mM potassium phosphate buffer (pH 5.0) immediately before use.

STUDY OF ATRAZINE DEGRADATION IN THE PRESENCE OF LACCASE

To assess changes in adsorption–desorption behavior of atrazine due to the possibility of atrazine degradation in the presence of enzyme, coincubation of atrazine and laccase was conducted in 50 mM potassium phosphate buffer

(pH 5.0) at 27±1°C. Atrazine concentration was 5 mg/L, laccase concentration and activity were 3.5 g/L and 10^{-6} M, respectively. After 24, 48, 75, 96, 120, 144, and 168 h of incubation solutions were sampled, filtered through cellulose filter with cutoff 5 kD, and subjected for atrazine analysis using high-performance liquid chromatography (HPLC).

ADSORPTION–DESORPTION EXPERIMENTS

Sorption–desorption experiments were conducted at six sorbate concentrations. The initial atrazine concentrations were 1, 2, 3, 5, 8, and 10 mg/L. Equilibrium time was determined as 24 h by preliminary experiments.

Adsorption experiments were conducted in plastic centrifuge tubes closed with caps. Tubes were first filled with 2 g of soil, and then 10 mL 50 mM potassium phosphate buffer (pH 5.0) was added. The tubes were shaken vigorously, placed on rotator Intelli-Mixer RM-2 (Elmi, Latvia), and left at 27±1°C for equilibration between soil and phosphate buffer. After 24 h, atrazine solution was added correspondingly with the scheme of experiments. If required, laccase solution was added to create final concentration of 3.5 g/L. Tree replicates were used for each initial atrazine concentration. Then tubes were shaken vigorously and placed on rotator for 24 h at 27±1°C. At the end of the equilibration period, the tubes were centrifuged at 1,200 g for 30 min to separate the solid and liquid phases. A 0.05-mL aliquot was removed from supernatant, filtered through cellulose filter with cutoff 5 kD, and subjected for atrazine analysis using HPLC.

Remaining atrazine solution was then removed with a pipette and replaced with clean potassium phosphate buffer for desorption. The tubes containing clean buffer were recapped and placed on the rotator at 27±1°C for atrazine desorption. After 24 h, the tubes were centrifuged and the supernatant was analyzed for desorbed atrazine using HPLC. The liquid was then removed, and the procedure was repeated until atrazine concentrations fell below the detection limit. In general, seven desorption steps were performed.

ATRAZINE ASSAY USING HPLC ANALYSIS

The determination of atrazine and its main metabolites desethylatrazine, desethyldesisopropylatrazine, 2-hydroxyatrazine, desethyl-2-hydroxyatrazine, and desisopropyl-2-hydroxyatrazine was conducted using HPLC technique as described elsewhere (Carabias-Martinez et al., 2002) with little modifications. HPLC was performed on a chromatograph Beckman Coulter System Gold (USA), equipped with two pumps, a membrane degasser, and a diode-array detector. The reverse phase C18 Ultrasphere ODS Beckman column (USA) 4.6 mm × 25 cm was used for separation. The diode array

detector was set at 210, 225, 230, and 245 nm. The mobile phase consisted of acetonitrile (solvent A) – 1 mM phosphate buffer at pH 7.0 (solvent B) linear gradient from 2% to 98% of solvent A in 35 min. Flow rate was 1 mL/min and the volume injected was 20 µL. The analytical column was kept at constant temperature 30°C.

Results and Discussion

LACCASE ACTIVITY IN SOILS USED

No laccase activity was detected under selected conditions. That was probably due to air-drying and long-term storage of soil samples. The obtained results allowed us supposing that only introduced laccase influenced on adsorption–desorption behavior of atrazine rather than inherent soil laccase.

DEGRADATION OF ATRAZINE IN THE PRESENCE OF LACCASE

After coincubation of laccase and atrazine, no changes in atrazine concentration were detected. Data on atrazine degradation in the presence of fungal laccase are given in Fig. 1.

As it can be seen from Fig. 1, atrazine concentration in the solution did not change all over the time of the experiment. At that, atrazine metabolites were not detected in the solution. That finding was evident for the fact that laccase did not induce atrazine degradation in the solution under selected conditions.

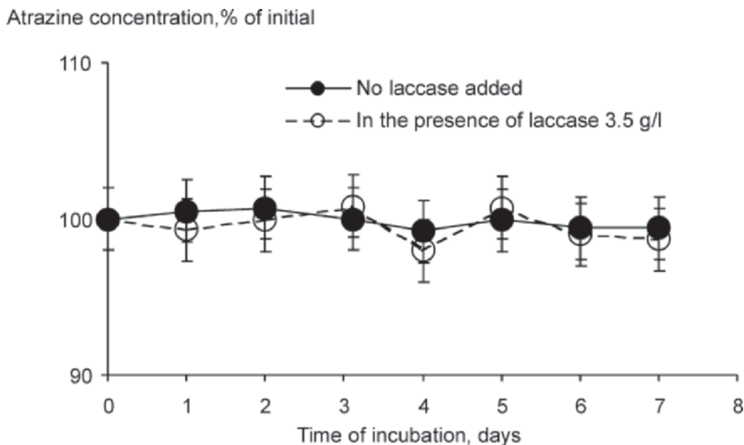

Fig. 1. Scavenging of atrazine in solution in the presence (empty markers) and absence (filled markers) of fungal laccase. Bars represent standard deviations

NONENZYMATIC ADSORPTION–DESORPTION OF ATRAZINE IN DIFFERENT SOILS

Adsorption–desorption isotherms for atrazine in different soils are presented in Fig. 2. A summary of results for atrazine adsorption–desorption data in different soils studied is presented in Table 2. Adsorption isotherms were not linear as indicated by the Freundlich n<1 in all the cases. The latter was indicative of adsorption by heterogeneous media where high energy sites were occupied first, followed by adsorption at lower energy sites (Weber et al., 1996).

Atrazine Freundlich adsorption constants K_F for studied soils varied in the range 0.81–5.54, which is in accordance with previously published data

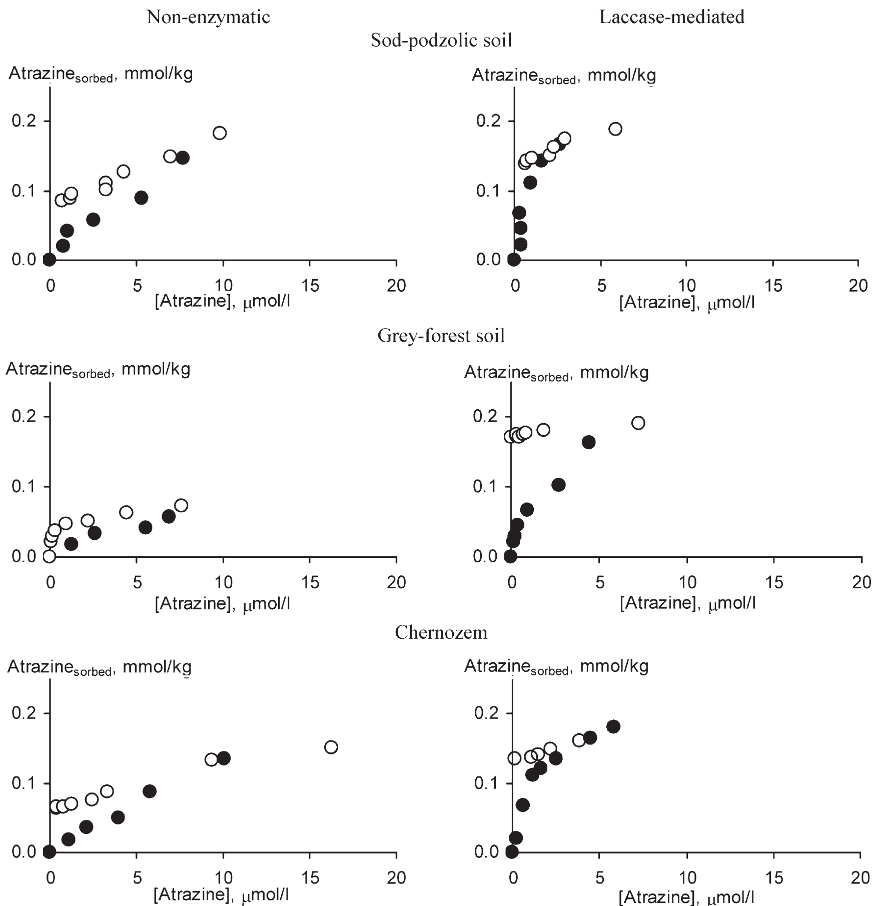

Fig. 2. Nonenzymatic and laccase-mediated adsorption (filled markers) and desorption (empty markers) of atrazine in three different soils

TABLE 2. Freundlich isotherm parameters (n and K_F) and hysteresis index H values for atrazine adsorption–desorption in different soils without fungal laccase addition

	Adsorption			Desorption			
Soil	K_F	n	R^2	K_F	n	R^2	H
Sod-podzolic	4.51	0.73	0.93	0.57	0.27	0.89	2.7
Gray forest	0.81	0.56	0.96	1.69	0.53	0.94	1.1
Chernozem	5.54	0.83	0.98	0.36	0.23	0.89	3.6

(Lesan and Bhandari, 2000). As a consequence of marginally higher organic carbon content, chernozem soil exhibited a larger sorption capacity for atrazine than other soils.

Desorption of atrazine from the soils was rather similar for sod-podzolic soil and chernozem while that was different significantly from gray forest soil where only negligible desorption hysteresis was observed. Desorption hysteresis was revealed in all soils with the chernozem soil manifesting a significantly greater hysteresis ($H = 3.6$) and gray forest soil exhibiting the smallest value of hysteresis ($H = 1.1$). The value of H close to 1 in case with gray forest soil reflected the fact that almost all adsorbed atrazine was readily desorbed. On the other hand, $H > 1$ was observed likewise for sod-podzolic and chernozem that indicated for partially irreversible adsorbed atrazine. The hypothesis on partial irreversible atrazine sorption could also be confirmed by the finding that no products of atrazine degradation were found over duration of the experiment. The latter meant that atrazine scavenging in solution resulted only from adsorption but not degradation process.

The gray forest soil was collected from the field site and contained freshly decaying biomass and consequently humic substances lacking in aromatic structures as compared with the other soils. This may be the reason why only negligible desorption hysteresis was observed in this case as hydrophobic binding between humic substances and atrazine was supposed to be a leading mechanism of their interaction.

LACCASE-MEDIATED ADSORPTION–DESORPTION OF ATRAZINE IN DIFFERENT SOILS

Adsorption–desorption data for atrazine in different soils in the presence of fungal laccase are presented in Fig. 2. A summary of results for atrazine adsorption–desorption behavior in different soils in the presence of fungal laccase is presented in Table 3. Similar to atrazine adsorption by soils without enzyme addition, isotherms in the presence of fungal laccase were not linear

TABLE 3. Freundlich isotherm parameters (n and K_F) and hysteresis index H values for atrazine adsorption–desorption in different soils in the presence of fungal laccase

	Adsorption			Desorption			
Soil	K_F	n	R^2	K_F	n	R^2	H
Sod-podzolic	5.80	0.61	0.74	0.36	0.13	0.93	4.7
Gray forest	3.13	0.56	0.99	0.21	0.02	0.74	28.5
Chernozem	6.80	0.66	0.86	0.22	0.06	0.68	10.9

either. The values of Freundlich n value varied in the range 0.56–0.66 being less than those obtained from adsorption experiments without laccase. The latter was indicative for complex adsorption process of atrazine by soils in this case rather than simple partitioning.

As it can be seen from Fig. 2 and Tables 2 and 3, laccase introduction resulted in a dramatic increase in atrazine adsorption. Atrazine Freundlich constants K_F for studied soils in the presence of fungal laccase varied in the range 3.13–6.80 exceeding those for adsorption experiments without enzyme.

Desorption of atrazine was also extremely reduced in the presence of fungal laccase. In the presence of enzyme atrazine was observed to desorb to a significantly greater extent than in case when laccase was not added, especially in soil where the lowest organic matter content was found. H values for atrazine adsorption mediated by laccase varied from 4.7 to 28.5, which exceeded those values 2–27 times for nonenzymatic adsorption. It appeared that adsorption of atrazine in all soils studied was enhanced when laccase was present. Like in case of nonenzymatic adsorption, no products of atrazine degradation were found over duration of the experiment. Therefore, enhanced laccase-mediated adsorption of atrazine in soils and increased hysteresis of that process could be attributed to the covalent bonding of atrazine to soil organic matter by oxidative coupling mechanism. This is an important observation as it points to the potential of laccase enzymes to effectively retard the mobility of atrazine in soils and groundwater.

Conclusions

The study was aimed to present and discuss results from ongoing studies evaluating the effects of fungal laccase in the fate of atrazine adsorption and desorption in soils. Adsorption–desorption of atrazine was studied in three soils with varying properties including organic matter types and contents. Effect of the addition of laccase enzyme on the adsorption and desorption behavior of atrazine was evaluated. It was observed that

adsorption isotherms for all cases were nonlinear, indicative of adsorption by heterogeneous media where high energy sites were occupied first, followed by adsorption at lower energy sites. Atrazine desorption in sod-podzolic soil and chernozem exhibited some hysteresis in the absence of laccase; gray forest showed little hysteresis (H = 1.1) for atrazine. Addition of fungal laccase resulted in dramatic increases in adsorption for all soils studied. Freundlich K_F values increased with laccase addition possibly as a result of enzyme-catalyzed oxidative coupling of atrazine to soil organic matter. Desorption was reduced to little or negligible: hysteresis, as reflected by the H values, was significantly enhanced upon enzyme addition. Hysteresis indexes derived from laccase-mediated adsorption experiments 2–27 times exceeded those values for nonenzymatic adsorption. Results of this study illustrate the capability of engineered humification processes, such as those catalyzed by laccase enzymes, to significantly alter the fate and transport of atrazine in soil, sediment, and groundwater systems.

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