Chapter 10

Mycoremediation of Atrazine in a Contaminated Clay-Loam Soil and its Adsorption-Desorption Kinetic Parameters

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Additional information is available at the end of the chapter

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Abstract

Clean-up of contaminated soils with atrazine is an ecological responsibility. The objectives of this study are to evaluate atrazine degradation in a clay-loam soil microcosm using fungal enzyme extracts from *Trametes maxima* and its co-culture with *Paecilomyces carneus* and to determine the kinetic parameters of the adsorption-desorption of atrazine in soil. Fungal co-culture extract (*T. maxima-P. carneus*) and monoculture (*T. maxima*) were able to degrade 100% of atrazine. However, we observed variation in atrazine degradation over the course of the evaluated time period, which suggests that an adsorption-desorption process is occurring in the soil. Adsorption-desorption kinetic parameters of the Freundlich model revealed that the studied soil has a significant capacity to adsorb atrazine (K_F = 8.2148; r^2 = 0.992 and *P*-value < 0.0001), while according to the desorption parameters (K_F = 5.4992; r^2 = 0.245 and *P*-value = 0.036) and hysteresis index (H = 0.573), the soil does not desorb atrazine at the same rate. Fungal enzyme extracts from a monoculture and co culture of *T. maxima* were able to degrade atrazine in a short time period (< 12 h). The ability of the contaminated soils to adsorb and desorb atrazine should be taken into account in mycoremediation systems.

Keywords: bioremediation, fungal enzyme extract, laccase, soil organic matter

1. Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is the most widely used herbicide around the world. In agricultural soils, approximately 29–34 million kg of atrazine are applied per year [1]. In Mexico, more than 45% of pesticides are categorized as herbicides, and atrazine is one of the most commonly used herbicides in Mexican agriculture [2]. Since 1975, atrazine has been applied to control broadleaf and grass weeds in agricultural crops, including corn, sorghum and sugar cane. Atrazine kills susceptible plants by binding to the quinone protein in photosystem II and inhibiting photosynthetic electron transport [3].

Atrazine is a pre-emergent herbicide and is considered to have low persistence in soil (<12 months). However, its low mobility in soil and its physical and chemical properties contribute towards the contamination of ground and surface waters, which represents a risk to the environment and to human health [4]. In Mexico, atrazine levels in water often exceed the maximum permissible levels for drinking water (0.1 μg L^{-1}) as established by Europe and by the health advisory board of the United States Environmental Protection Agency (EPA) [5, 6]. At a molecular level, atrazine has distinct fates in the environment and may be found in soil, water, biomass (plants) or air. In soil, atrazine is adsorbed by clay particles; however, other adsorption-desorption processes may be involved in its translocation in plants, movement in soil and mobility in aqueous systems, as well as its eventual abiotic or biological degradation [7].

The clean-up of soils contaminated with atrazine is an ecological responsibility, and the discovery of a safe and economical method is a major priority for land management agencies [8]. One such possibility is mycoremediation, or the use of fungal organisms and their enzymes to degrade or transform environmental pollutants [9]. This strategy has been used to degrade pesticides [10], aromatic and polycyclic hydrocarbons [11] and endocrine disruptors [12]. The degradation of environmental pollutants by fungi, specifically by white-rot fungi, is due to their ability to synthesize ligninolytic enzymes, such as laccase, manganese peroxidase and lignin peroxidase, as well as their production of hydrogen peroxide [13, 14].

However, mycoremediation faces several challenges in order to improve the feasibility of this strategy. The following issues, for example, should be addressed: (i) the competition/proliferation of native soil microorganisms (actinomycetes and bacteria) may inhibit the growth of bioremedial fungi; (ii) bioremedial fungi have a limited capacity to produce ligninolytic enzymes. Enzyme production varies depending on the strain and species and is mainly influenced by the content and availability of nutrients (carbon, nitrogen, metal ions, etc.), which stimulate fungal growth and the synthesis of ligninolytic enzymes; finally, (iii) edaphic and environmental factors may adversely affect the establishment and growth of bioremedial fungi [9].

The use of fungal extracts with a proven high activity of ligninolytic enzymes is one means of improving the degradation of pollutants in soil, which may also address some of the aforementioned challenges. Ligninolytic enzymes in white-rot fungi, for example, may be enhanced through the use of fungal co-cultures, although the mechanism by which increased enzyme

activity occurs has not yet been described [15]. Given this context, the objectives of the study were: (i) to evaluate the degradation of atrazine in soil microcosms by a white-rot fungus (*Trametes maxima*) and its co-culture with a soil-borne micromycete (*Paecilomyces carneus*) and ii) to determine the absorption-desorption kinetics of atrazine in a clay-loam soil.

2. Materials and methods

2.1. Fungal source and molecular identification

The white-rot fungi *T. maxima* was isolated from a carpophore collected in a rain forest (19°32′21.23″ N, 97°00′47.29″ W) near Vega del Pixquiac, San Ándres Tlalnelhuayocan, Veracruz, Mexico. To obtain the isolate, 0.5–1 cm fragments of the carpophore were cut and removed; these were washed in ethanol (70%) for 1 min, in sodium hypochlorite (50%) for 3 min and finally, in sterile, distilled water. The washed and disinfected fragments were placed on potato-dextrose agar plates (Bioxon®, Mexico) and supplemented with chloramphenicol (20 mg/L; Sigma-Aldrich, St. Louis, MO, USA) to prevent bacterial contamination and benomyl (3 mg/L; Biesterfeld Co., Mexico) to inhibit mold growth.

The soil-borne micromycete *P. carneus* Duché & R. Heim (Trichocomaceae: Ascomycota) was donated by the Micromycetes Laboratory of the Institute of Ecology (INECOL A.C.) located in Xalapa, Mexico. This strain was isolated from an andic acrisol soil (texture: loam-silt loam) from a coffee plantation in Huatusco, Veracruz, Mexico (location: 19°12′57″ N, 96°53′7″ W). The carpophores of *T. maxima* (Mont.) A. David & Rajchenb (Polyporaceae: Basidiomycota) and the *P. carneus* strain are stored in the herbarium (XAL) and Micromycetes Culture Collection of INECOL. Both strains were maintained and subcultured in potato dextrose agar.

2.2. Soil sampling and characterization

Soil samples were collected from the first horizon of <20 cm profundity at a sugar cane plantation in Mahuixtlan, Veracruz, Mexico (location: 19°23′21.3″ N, 96°53′34.9″ W). Plant residues and rocks were removed manually. Soil was sieved in 2 mm mesh in the laboratory and dried at 20°C for 5 days prior to use. The physical and chemical characteristics of the soil were determined using standard methods to establish texture (clay loam soil), soil organic matter (4.35%), pH (4.86), NH_4-N (5.8 mg kg^{-1}), soluble salts (5.38 S m^{-1}), acidity (0.053 meq 100 g^{-1}), cation exchange capacity (16.41 meq 100 g^{-1}), water holding capacity (WHC) (53.6%) and electrical conductivity (53.75 μS cm^{-1}).

2.3. Production of ligninolytic enzymes through fungal co-culture

Modified Sivakumar culture medium [16] was used to produce laccase, MnP and H_2O_2 for the monoculture of *T. maxima* and the co-culture of both *T. maxima* and *P. carneus*. To establish the co-culture, four agar plugs of *T. maxima* (7 days old) were deposited in a 250 mL Erlenmeyer flask with 120 mL of modified Sivakumar culture medium. After 3 days, four agar plugs of *P.*

carneus (9 days old) were added. Monocultures of both fungi were established at the same time. Fungal cultures were incubated at 25°C and 120 rpm for 6 days. After this step, the fungal enzyme extracts (FEEs) were centrifuged at 7000 rpm during 10 min. The supernatant was filtered with a 0.2 mm nylon filter; this process allows a cell-free extract to be obtained, which was used to determine laccase and MnP activity and H_2O_2 content.

2.4. Ligninolytic enzyme activity and H_2O_2 quantification

2.4.1. Laccase determination

Laccase activity was determined according to More et al. [17] by measuring the oxidation of ABTS [2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)] in a reaction mixture (1 mL) containing 100 μL of ABTS (0.5 mM, Sigma, St. Louis, MO, USA), 800 μL of acetate buffer (100 mM, pH 4.5) and 100 μL of enzyme extract. Absorbance changes in the presence of the enzyme were monitored during 5 min at 420 nm ($\varepsilon = 3.6 \times 10^4\ M^{-1}\ cm^{-1}$). One unit of laccase activity was defined as the amount of enzyme required to oxidize 1 μmol ABTS per minute per milligram of protein under the assay conditions.

2.4.2. Manganese peroxidase assay

MnP activity was determined at 610 nm ($\varepsilon = 4460\ M^{-1}\ cm^{-1}$), following the method described by Kuwahara et al. [18]. The reaction mixture contained the following: 700 μL of enzyme extract, 50 μL of phenol red (0.2%), 50 μL of sodium lactate (0.5 mM), 50 μL of egg albumin (0.1%), 50 μL of manganese sulfate (2 mM) and 50 μL of H_2O_2 (2 mM). The reaction was carried out in 50 μL of sodium succinate buffer (20 mM) at pH 4.5. After 5 minutes, 50 μL of NaOH (2N) was added to stop the reaction. One enzyme unit was defined as 1 μmol of the product formed per minute per milligram of protein under the assay conditions.

2.4.3. Hydrogen peroxide content

H_2O_2 content of the fungal enzyme extracts (FEEs) was determined using the iodide/iodate method, according to Klassen et al. [19]. Three milliliters of the FEEs were mixed with 3 mL of a solution containing KI (33 g), NaOH (1 g) and $(NH_4)_6Mo_7O_{24} \times 4H_2O$ (0.1 g) in 500 mL of distilled, deionized water, in addition to 3 mL of a solution containing $C_8H_4KO_4$ (10 g) in 500 mL of distilled, deionized water. The absorbance of the resulting solution was measured at 351 nm in a 3 cm^3 cuvette. The blank absorbance was determined by substituting the FEEs with a sterile Sivakumar culture medium in the reaction mixture. Hydrogen peroxide content was calculated by substituting with H_2O_2 reagent (30%, J.T. Baker™) according to the standard curve of the absorbance of known concentrations (0.0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg L^{-1}).

2.5. Biodegradation studies

Biodegradation of atrazine was evaluated in sterile soil microcosm conditions. First, 20 g of air dried soil was placed in serological flasks (100 cm^3). Then, the sterile soil was contaminated

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with atrazine (Sigma-Aldrich Co., USA) at the field application rate of 5mg/kg [8], and 20 mL of methanol were added (analytical grade, Honeywell Burdick & Jackson, Muskegon, MI, USA). Soil-methanol-atrazine was mixed using a sterile spatula until the complete evaporation of methanol under a laminar flow hood.

Three treatments were evaluated: *T. maxima* extract, *P. carneus* extract and their co-culture extract (*T. maxima-P. carneus*). Soil microcosms were adjusted to a water holding capacity (WHC) of 40% using 0.215 mL of fungal extract per gram of soil. Four experimental units (serological flasks) were used per treatment. Atrazine degradation was evaluated at 1, 3, 6 and 12 h using high-pressure liquid chromatography (HPLC) analysis. In addition, abiotic (sterile soil) and biotic (nonsterilized soil) controls were used.

2.6. Adsorption-desorption studies

Experiments were conducted using six sorbate concentrations of atrazine (0.5, 1, 5, 10, 20 and 30 mg/kg). Two grams of all soil samples were added to a polypropylene bottle (20 mL), and immediately 5 mL of a methanol solution with the sufficient amount of atrazine was added to obtain the established concentration. Bottles were shaken vigorously (24 h) and placed on a flat rotator shaker (120 rpm) at room temperature (27 ± 1°C) [20]. Four replicates were used for each initial concentration of atrazine. After an equilibration period (24 h), samples were centrifuged in cold (5°C) at 7000 rpm during 20 min. Then, 0.2 mL of supernatant was filtered through a 0.22 μm nylon syringe. The filtrate was used to analyze the atrazine adsorbed using HPLC.

Desorbed atrazine was determined by examining the solid phase of the centrifuged samples; 5 mL of methanol was added in each bottle and shaken during 24 h at 120 rpm in a flat rotatory shaker. After the agitation period, the bottles were centrifuged and filtered as mentioned above for further atrazine analysis.

2.7. Atrazine analysis

The analysis of atrazine degradation and its desorption-adsorption was performed using a Thermo-Scientific HPLC system coupled to a diode array detector (SpectraSystem UV8000), a sampling injector (SpectraSystem AS3000) and a pump (SpectraSystem P4000) equipped with a Restek ultra C18 column (5 mm × 150 mm × 4.6 mm). The column was operated at 25°C with a flow rate of 1.0 mL min^{-1} and an injection volume of 20 μL. An isocratic mobile phase was established using acetonitrile-water at a ratio of 70:30. The HPLC-photodiode array detector was monitored at 215 nm [8]. The HPLC method had a running time of 10 min and a retention time of 3.8 min, which enabled the detection and quantification of atrazine. The atrazine detection limit was 0.05 mg g $soil^{-1}$. The standard curve for atrazine [atrazine = (peak area – 491818)/804962] was made using a standard analytical solution (Sigma-Aldrich Co., USA) at different concentrations, and the r^2 value was >0.99. The extraction efficiency of this method was 105%, and this value was taken into account in the final quantifications.

3. Results and discussion

3.1. Enzyme characterization of fungal extracts

Laccase activity and H_2O_2 production in the fungal co-culture (laccase = 18956.0 U/mg of protein and H_2O_2 = 6.2 mg/L) were significantly higher (T = 6.19, P = 0.0004) than in the *T. maxima* monoculture (laccase = 12866.2 U/mg of protein and H_2O_2 = 4.2 mg/L). Regarding MnP activity, we did not find significant differences between the fungal co-culture and the *T. maxima* monoculture (T = 0.27, P = 0.3957). Since *P. carneus* is a soil microfungus (Hyphomycete), it did not show laccase or MnP activity; only H_2O_2 production (0.9 mg/L) was detected, which was significantly lower (F = 126.4, P = 0.00001) than in the *T. maxima* monoculture (4.2 mg/L) and fungal co-culture (6.2 mg/L, **Table 1**).

	Fungal enzyme extracts			
Variable	***P. carneus***	***T. maxima***	**Co-culture**	**Mean Comparison test**
Laccase (U/mg of protein)	ND	12866.2 ± 446.7	18956.0 ± 204.0	*t*-student [T = 6.19, P = 0.0004]
MnP (U/mg of protein)	ND	572.4 ± 31.8	542.6 ± 43.5	*t*-student [T = 0.27, P = 0.3957]
H_2O_2 (mg/L)	0.9 ± 0.07 c	4.2 ± 0.10 b	6.2 ± 0.15 a	Fisher [F = 126.4, P = 0.00001]

Laccase and MnP were compared with the *t*-student test, and H_2O_2 content was compared using an ANOVA and LSD test for mean comparison. Means with different letters are significantly different from each other (P = 0.05). ND = No detected.

Table 1. Amount of enzymes in fungal extracts.

Laccase is an important enzyme in white-rot fungi; this enzyme is a defence mechanism against saprotrophic and parasitic microfungi. This phenomenon has been reported for *Lentinula edodes* [21], *Agaricus bisporus* [22] and *Pleurotus ostreatus* [23] when infected with *Trichoderma* sp. These macromycetes have been studied due to their importance as edible mushrooms, and *Trichoderma* is their naturally antagonistic fungus, especially in production systems. In particular, recent studies have sought solutions stemming from fungal interactions to obtain relevant biotechnological solutions and products. Thus, the interaction between white-rot fungi (Basidiomycetes) and other soil-borne micromycetes (hyphomycetes) has received greater interest in recent years [24, 25].

One of the principal applications of fungal co-cultures is to increase ligninolytic enzyme activity (laccase, MnP and LiP), and these may then be applied to resolve environmental problems, such as the contamination of soil and water with pesticides or the presence of endocrine disruptors, medical drugs, hydrocarbons, dyes or other emerging contaminants in the environment. Several studies have reported that soil-borne micromycetes enhance

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ligninolytic enzyme activity in white-rot fungi; for example, Baldrian [25] reported that *Sphaerospermum* sp., *Acremonium* sp., *Fusarium reticulatum*, *Humicola grisea* and *Penicillium rugulosum* enhanced laccase activity in *Trametes versicolor* and *Pleurotus ostreatus* when co-cultivated. Dwivedi et al. [26] reported an increase in the laccase activity of *Pleurotus ostreatus* when co-cultured with *Penicillium oxalicum*. In addition, Chan-Cupul et al. [15] recently demonstrated that laccase and MnP activity in a specific co-culture may be increased if the culture media are optimized. In that study, a 1.8- and 2.9-fold increase in laccase and MnP activities, respectively, was recorded for the co-culture of *T. maxima* and *P. carneus*.

3.2. Biodegradation studies

Figure 1 shows atrazine degradation by fungal enzyme extracts (FEEs) from the monocultures of *T. maxima* and *P. carneus* and their co-culture. One hour after application, the co-culture enzyme extract degraded 100% of atrazine at a significantly higher rate ($F = 331.31$, $P = 0.00001$) than *T. maxima* and *P. carneus* extracts, which degraded 80.0% and 27.3% of atrazine, respectively (**Figure 1A**). At 3 h after application, the monoculture extract of *T. maxima* (84.5%) statistically achieved the same level of atrazine degradation as the co-culture extract (89.1%); however, both values were higher ($F = 320.5$, $P = 0.0001$) than atrazine degradation by the *P. carneus* enzyme extract (5.3%, **Figure 1B**).

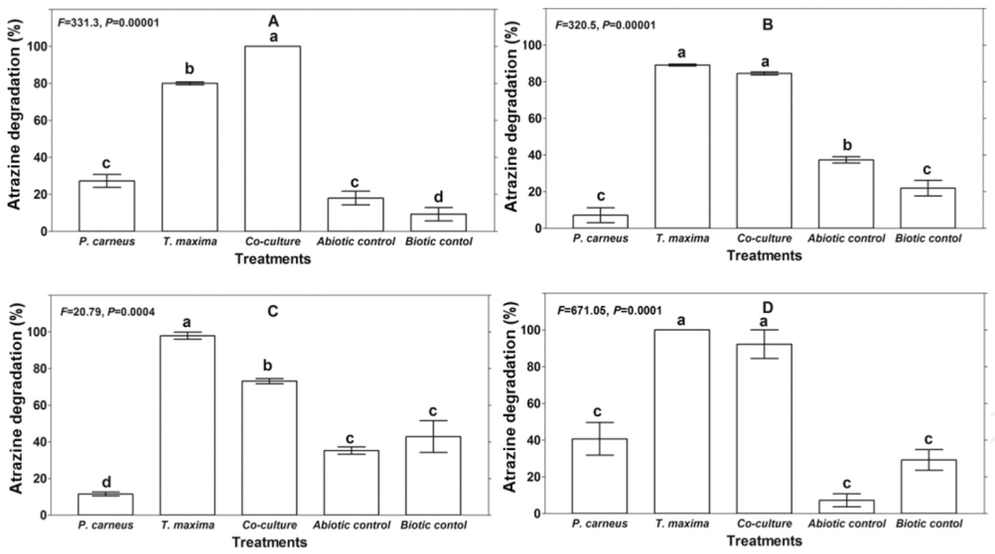

Figure 1. Atrazine degradation at 1 h (A), 3 h (B), 6 h (C) and 12 h (D) after application of fungal enzyme extracts in a clay-loam soil. Bars (mean ± standard error) with different letters are statistically different from one another (LSD test $P = 0.05$).

At 6 h after application (**Figure 1C**), the relationship of *T. maxima* and its co-culture with *P. carneus* was inverted. Atrazine degradation by the co-culture enzyme extract decreased by 23.9% in comparison to its initial rate of degradation (at 1 h). This may be attributed to the absorption of atrazine by the soil, which motivated the investigation of the kinetic absorption-desorption parameters of atrazine in the studied clay-loam soil. Meanwhile, degradation of

atrazine by the fungal monoculture extract increased to 97.9% (6 h), and *P. carneus* showed the lowest percentage of degradation (8.9%; $F = 20.79$, $P = 0.0004$).

However, during evaluation the degradation of atrazine by the fungal co-culture enzyme extract increased once again (92.2% at 12 h). This may be due to a desorption effect of atrazine previously absorbed by soil particles, principally clay. Meanwhile, the *T. maxima* monoculture extract degraded 100% of atrazine by this time, and the *P. carneus* extract also reached its maximum level of atrazine degradation (40.7%). At the end of evaluation period (12 h), both the *T. maxima* extract and its co-culture with *P. carneus* degraded 100% of atrazine. However, the increase in degradation by the *P. carneus* extract was not significant and did not reach levels of greater than 25% ($F = 671.05$, $P = 0.0001$, **Figure 1D**).

During mycoremediation, a single strain is commonly used. The application of bioremedial fungi in the soil is often based on the inoculation of immobilized mycelium in organic substrates, such as pine sawdust, wood chips, peat, corn cobs, wheat straw, bark, rice grains, sugarcane bagasse, coffee pulp or sugar beet pulp [27–30]. However, this technology has several challenges to overcome, which are as follows: (i) the competition and proliferation of native soil microorganisms (microfungi, bacteria and actinomycetes) with bioremedial fungi [9]; (ii) the limited capacity of inoculated fungi in the soil to produce sufficient amounts of the ligninolytic enzymes responsible for degrading contaminants [31–33]; (iii) the adverse effects of environmental and edaphic conditions on the establishment or growth of bioremedial fungus [14] and (iv) the amount of contaminants in the soil, which in some cases may be toxic to the bioremedial fungi [14].

One alternative for overcoming these challenges is the use of fungal enzyme extracts produced in fungal co-culture systems, which may enhance the amount of ligninolytic enzymes [34]; these extracts may then be applied to soil through irrigation systems by drenching or by immobilizing ligninolytic enzymes in chitosan, alginate or nanoparticles [35]. In our study, we applied fungal enzyme extracts from a co-culture to degrade atrazine in a clay-loam soil and found efficiencies of 100% at 6 and 12 h. Other studies have reported the ability of white-rot fungi extracts to degrade atrazine. For example, *Phanerochaete chrysosporium* extract can degrade atrazine in the soil microcosm (38% at 8 days), although its volumetric enzyme activity is low (MnP = 77.6 U/L, LiP = 149 U/L), as this species has low or null laccase activity [32]. In batch studies, Pereira et al. [36] reported that 39% of atrazine was degraded using a broth culture of *Pleurotus ostreatus* INCQ40310; the rate of degradation was enhanced to 71% when the broth culture was optimized by manipulating the nutritional compounds of the culture medium.

Several additional studies have used fungal co-cultures or their products, such as ligninolytic enzymes, to degrade contaminants. Recently, Pan et al. [37] demonstrated the feasibility of the fungal co-culture extract between *Coprinopsis cinerea* and *Gongronella* sp. to decolorize indigo dye. However, the native laccase from the fungal extract did not degrade indigo dye, and it was necessary to add ABTS (2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonic acid)) as a redox mediator to degrade 75% of the dye. In another study, Qian and Chen [38] reported that the crude extract from the co-culture of *T. versicolor* and *Phanerochaete chrysosporium* degraded 20% more benzo-α-pyrene than the crude extracts of the monocultures of both fungi.

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3.3. Atrazine absorption-desorption in a clay-loam soil

Figure 2 shows the adsorption-desorption of atrazine in the studied clay-loam soil. Depending on the concentration of atrazine dissolved in soil, between 39% and 77% is absorbed (**Figure 2A**). More atrazine is adsorbed than desorbed, or in other words, the desorption of atrazine is slower than its adsorption given the studied the soil type and time period (24 h, **Figure 2B**). Atrazine desorption is slower when high concentrations are adsorbed by the soil; i.e., when 20 mg/L was absorbed, only 1% was desorbed at 24 h. In this sense, Davidchik et al. [39] suggest that the adsorption of atrazine may be irreversible if a high concentration is found in the soil; these authors consider that oxidative binding is the most probable mechanism of atrazine incorporation into the organic matter.

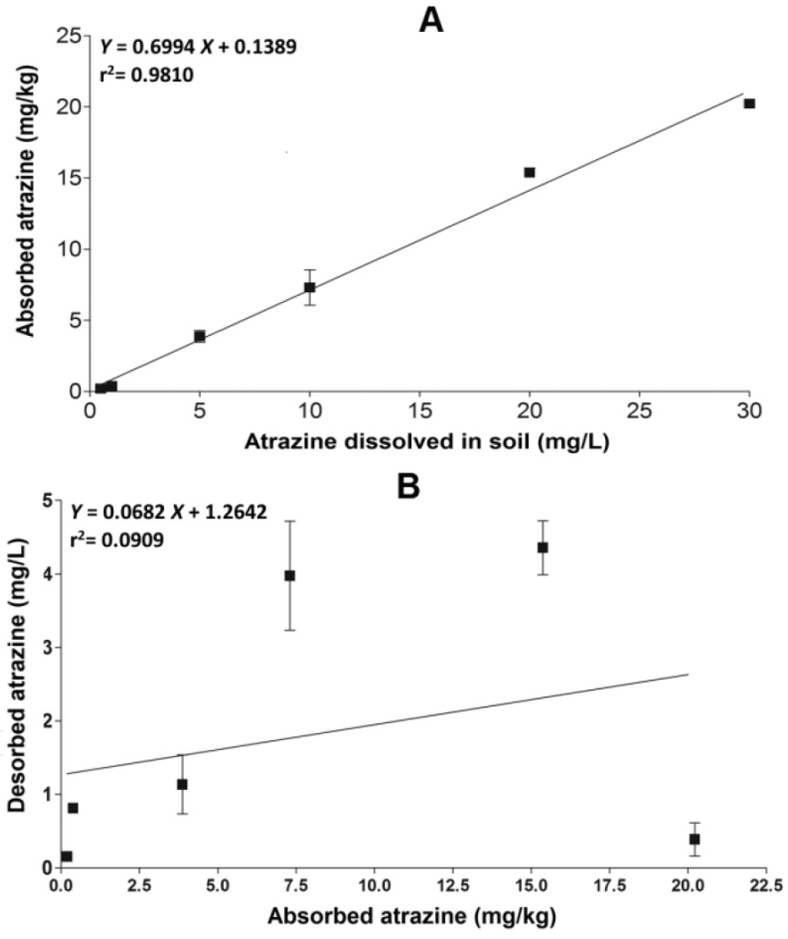

Figure 2. Adsorption (A) and desorption (B) of atrazine in a clay loam soil.

Adsorption and desorption values were linearized using the Freundlich equation (Eq. (1)), where q_e is the amount adsorbed at equilibrium (mg of atrazine/g of soil) and C_e the equilibrium concentration of atrazine in the solution (mg of atrazine/L). **Figure 3** shows the linearized Freundlich isotherms for atrazine adsorption and desorption, while **Table 2** describes the Freundlich isotherm parameters and hysteresis index.

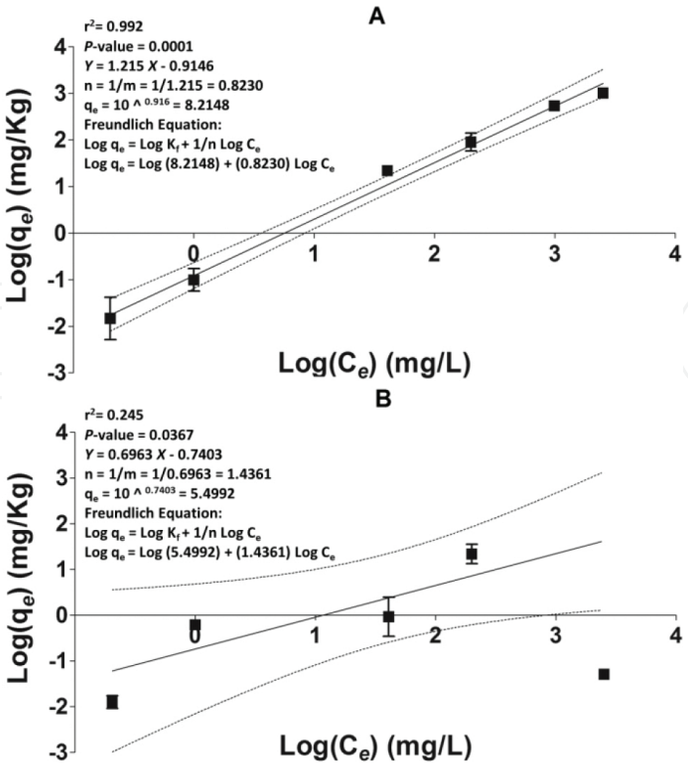

Figure 3. Linearized Freundlich isotherms for atrazine adsorption (A) and desorption (B) from a clay-loam soil.

Soil	Adsorption				Desorption				
	K_F	*n*	r^2	*P*-value	K_F	*N*	r^2	*P*-value	H
Clay-loam	8.2148	0.8230	0.992	0.0001	5.4992	1.436	0.245	0.036	0.573

K_F = Freundlich adsorption-desorption constant; *n* = absorbent constant; r^2 = regression coefficient; H = hysteresis index.

Table 2. Freundlich isotherm parameters and hysteresis index values for atrazine adsorption-desorption in a clay loam soil.

$$\log q_e = \log K_F + 1/n \log C_e \quad (1)$$

The Freundlich constant for adsorption of atrazine was 8.2148, which was higher than that reported by Kulikova et al. [7], who studied the absorption of atrazine to three soils with different textures (silt-loam: sod-podzolic [K_F = 4.51] and gray forest [K_F = 0.81] and clay-loam: chernozem [K_F = 5.54]). These authors suggest that clay-loam soil has high levels of organic carbon (organic matter), which leads to a high rate of atrazine absorption. In our study, the soil also possessed this characteristic, as a high organic matter content (4.35%) was detected in the soil analysis due to the incorporation of crop residues (sugarcane stalks) to the soil. In another study, Naga-Madhuri et al. [40] reported a lower K_F (=2.66) for atrazine adsorption in a silty clay-loam soil; the authors suggested that this value is high and may be due to the high electric conductivity and organic matter content of the studied soil.

On the other hand, the Freundlich desorption constant for atrazine was lower (K_F = 5.4992) than the adsorption constant (K_F = 8.2148). This was reflected in the hysteresis value (H = 0.573), which has a maximum value of 1; in this case, values near 1 indicate that almost all adsorbed atrazine is readily desorbed [7]. In this study we found that the clay-loam soil used in mycoremediation experiments does not desorb the adsorbed atrazine to a great extent, due to the high organic carbon content of the soil. Future studies will need to further examine the effect of enzyme extracts from fungal co-cultures and the adsorption-desorption phenomenon of atrazine in contaminated and bioremediated soils.

4. Conclusions

We conclude that:

1. The co-culture of *T. maxima* and *P. carneus* increases laccase activity and H_2O_2 content in the fungal enzyme extract.
2. Both the fungal enzyme extract of the monoculture of *T. maxima* and its co-culture with *P. carneus* were able to degrade atrazine in a short period of time (12 h) in a contaminated clay loam soil at a field application rate of 5 mg/kg.
3. Atrazine was highly adsorbed by the studied clay-loam soil. This was reflected by its high Freundlich coefficient for adsorption and low coefficient for desorption.

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