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Effects of combination of plant and microorganism on degradation of simazine in soil

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Abstract

The degradative characteristics of simazine (SIM), microbial biomass carbon, plate counts of heterotrophic bacteria and most probably number (MPN) of SIM degraders in uninoculated non-rhizosphere soil, uninoculated rhizosphere soil, inoculated non-rhizosphere soil, and inoculated rhizosphere soil were measured. At the initial concentration of 20 mg SIM/kg soil, the half-lives of SIM in the four treated soils were measured to be 73.0, 52.9, 16.9, and 7.8 d, respectively, and corresponding kinetic data fitted first-order kinetics. The experimental results indicated that higher degradation rates of SIM were observed in rhizosphere soils, especially in inoculated rhizosphere soil. The degradative characteristics of SIM were closely related to microbial process. Vegetation could enhance the magnitude of rhizosphere microbial communities, microbial biomass content, and heterotrophic bacterial community, but did little to influence those community components responsible for SIM degradation. This suggested that rhizosphere soil inoculated with microorganisms-degrading target herbicides was a useful pathway to achieve rapid degradation of the herbicides in soil.

Key words: bioremediation; degradation; rhizosphere; simazine (SIM)

Introduction

Pesticides have become an integral part of today's intensive agriculture. Widespread and large-scale use of pesticides during the past five decades or more has led to global problem of pollution of soil and water resources (Wehtje *et al*., 1983; Spliid and Kopper, 1998). Simazine (SIM) was a commonly used higher selective triazine herbicide to control annual broadleaf weeds and some annual grasses. SIM is of concern to doing harm to second season crop due to higher residual activity and the ground water quality affected by leaching (Albarrán *et al*., 2003; Neera *et al*., 2004). SIM is relatively an immobile organic compound that represents common industrial and agricultural pollutants with moderate-to-high soil sorption coefficients (Fang *et al*., 2001). SIM degrades relatively slowly in soil and is a persistent pollutant in agricultural soil. However, little information on biodegradation of SIM and the bioremediation of its pollution can be found. It is obviously important to examine the biodegradation rate of SIM in agricultural soil due to its persistence and residual harmful effects to crops and animals.

Vegetation has a decisive effect on the fate of soil applied pesticides and organic contaminants often disappear quickly from the planted soil compared to unplanted soil (Walton *et al*., 1994; Cunningham *et al*., 1996; Yu *et al*., 2003). Microbial transformation/mineralization might be the most important route for pesticide degradation in soils. The size and the activity of the soil microbial biomass affect the rate of pesticide degradation. Plants sustain large microbial populations in the rhizosphere by secreting substances, such as carbohydrates and amino acids, through root cells and by sloughing root epidermal cells. The magnitude of rhizodeposition by plants can be quite large. Root cap cells may be lost to the soil at a rate of 10000 cells per plant per day. In addition, root cells secrete mucilage, a gelatinous substance that is a lubricant for root penetration through the soil during growth (Damaj and Ahmed, 1996; Dandurand *et al*., 1997; Holden and Firestone, 1997). This mucigel produces root exudates. Soluble exudate includes aliphatic and aromatic hydrocarbons, amino acids, and sugars. Root cap cells and exudates provide important sources of nutrients for microorganisms in the rhizosphere. Larger microbial populations can, therefore, exist in the rhizosphere soil than in bulk soil. These larger populations have been shown to increase the degradation of organic chemicals including pesticides (Reilley *et al*., 1996; Nicholes *et al*., 1997; Joergensen, 2000; Molina *et al*., 2000; Steer and Harris, 2000).

Therefore, a study was conducted to evaluate the biodegradation characteristic of SIM and whether vegetation can promote the degrading microbial community in *Pennisetum* rhizosphere soil and bulk soil with uninoculated or inoculated SIM degrading bacterial community, further to explore effective approaches for the minimization of SIM residues in soil, and examine possible

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relationships between contaminant degradation rates and the structural of microbial communities in plant rhizosphere.

1 Materials and methods

1.1 Microorganisms

The bacterial community, designated *Agrobacterium* sp. R1 and used as the inoculant in this study, was isolated from a long-term (approx.15 years) contaminated soil from a previous pesticide storage facility. It can use SIM as its sole source of C and energy. Before inoculation, the bacterial community *Agrobacterium* sp. R1 was inoculated into a series of 250-ml flasks containing 100 ml of the medium consisting of 1 g of peptone, 0.5 g of beef extract, 0.5 g of NaCl in 1000 ml of water at pH 6.8. The culture was incubated for 5 d at 30°C on a rotary shaker (150 r/min). The bacterial community *Agrobacterium* sp. R1 was then inoculated into the soil at the ratio of 1.2×10^5 CFU/g soil.

1.2 Soil

Soil was collected from agricultural fields planted with rice at Huajia Campus, Zhejiang University. The soil was analyzed using standard methods and was found to have the following physical properties: organic matter, 19.92 g/kg, pH 5.62, cation exchange capacity, 14.59 cmol/kg, and sand, silt, and clay content of 65%, 29% and 6%, respectively. A bulk quantity of the soil was divided into four groups, each group comprised of three pots, and soil in each pot was 500 g. In groups one and two, soil was inoculated with bacterial community *Agrobacterium* sp. R1 at the ratio of 1.2×10^5 CFU/g soil. In groups three and four, soil was uninoculated. *Pennisetum* which could survive on the SIM contaminated soil was only planted in group one and group four. After treatment/planting, soil was maintained at 60% water holding capacity and plastic cups were kept in the glass house under natural day and night conditions for two months. There was no difference of *Pennisetum* growth between group one and group four.

At harvest, the rhizosphere soil from each pot was gently separated from the roots, and stored at 4°C, and used for microbiological analyses within 14 d. Because of the high rooting density in the pots, all the recovered soil was considered as the rhizosphere soil. The microbial biomass carbon, most probable number (MPN) of SIM degrading bacteria, and heterotrophic microbial population (by plate count), were determined for planted and non-planted soils as described below. Both rhizosphere and non-rhizosphere soil were transported immediately to the laboratory and prepared within a few hours and passed through a sieve of 20 mesh (0.9 mm).

1.3 Degradation of SIM

Fifty grams of the prepared rhizosphere soil or non-rhizosphere soil were weighed into a series of 250-ml flasks, and the solution of SIM was added at 20 mg/kg soil, and mixed well with the soil. The soil moisture was adjusted by the addition of water to 80% of its water-holding capacity. The soils were incubated on the day 1, 3, 5, 7, 14, 21, 28, 35, respectively, at 30°C in dark. At intervals of the day 1, 3, 5, 7, 14, 21, 28, 35, SIM was extracted and determined by high performance liquid chromatography (HPLC, LC-6A Shimadzu, Japan). All tests were triplicated.

1.4 Analysis of SIM

Residues in 10 g soil samples (planted and unplanted) were extracted with 50 ml of HPLC grade acetonitrile by shaking the suspension on a rotary shaker for 1 h. Soil suspension was centrifuged at 6000 r/min for 15 min and supernatant was transferred to a 250-ml volumetric flask. The soil was re-extracted with 50 ml portions of acetonitrile and a total of four extractions were performed for each sample. After extraction, the supernatants were pooled in a volumetric flask and volume was made up to 250 ml using acetonitrile. The residues of SIM were estimated using HPLC fitted with a UV detector. SIM was separated on a 12.5-cm LiChrospherw RP-18 endcapped column. The operating condition was: mobile phase, acetonitrile-water (30:70); wavelength, 210 nm; flow rate 1 ml/min.

1.5 Enumerations

Culturable heterotrophic bacteria were enumerated by a plate count technique. Serial dilutions of rhizosphere and non-planted soil were prepared in sterile phosphate-buffered saline (PBS) solution (pH 7), and appropriate dilutions were spread onto a medium containing (/L) 3 g trypticase soy broth (BBL) and 15 g agar. The PBS solution contained (/L): 8.0 g NaCl, 0.2 g KCl, 120 mg KH_2PO_4, and 91 mg K_2HPO_4. The media were incubated at 25°C in the dark for approximately one week prior to counting. Plates having between 30 and 300 colonies were counted.

1.6 Microbial biomass carbon and MPN of SIM degrading bacteria

Microbial biomass carbon in rhizosphere and non-rhizosphere soils was quantified using the fumigation extraction technique (Vance *et al*., 1987). In the degradation study, 10-fold soil dilutions were prepared (10^{-1}–10^{-7}) and three replicate tubes per dilution were used to determine MPNs for SIM degrading bacteria. SIM was the sole C and N source in the media. Each tube was inoculated with 1 ml of soil inoculum. The theoretical detection limit, based on the dilutions used, was 10 cells/g soil.

1.7 Statistical analysis

All the experimental data were processed using Microsoft Excel 2000. Differences between mean values were evaluated by a one-way analysis of variance (ANOVA), followed by the Student-Newman-Keuls-test of significant differences. Comparison of multiple means was performed using the Duncan's multiple range test with $p = 0.05$ using the SPSS program package (SPSS 11.5).

2 Results

Degradative characteristics of SIM in uninoculated non-rhizosphere soil, uninoculated rhizosphere soil, inoculated non-rhizosphere soil, and inoculated rhizosphere soil at initial concentrations of 20 mg/kg are shown in Fig.1. It could be observed that all the degradations fitted first-order kinetics. The corresponding kinetic data fitted first-order kinetics are listed in Table 1. At the initial concentration of 20 mg SIM/kg soil, the half-lives of SIM in uninoculated non-rhizosphere soil, uninoculated rhizosphere soil, inoculated non-rhizosphere soil, and inoculated rhizosphere soils were measured to be 73.0, 52.9, 16.9, and 7.8 d, respectively. It indicated that the degradative rates of SIM were in the order of uninoculated non-rhizosphere soil < uninoculated rhizosphere soil < inoculated non-rhizosphere soil < inoculated rhizosphere soil. There are slower degradative rates in uninoculated non-rhizosphere soil and uninoculated rhizosphere soil, and the highest degradative rate was observed in inoculated rhizosphere soil.

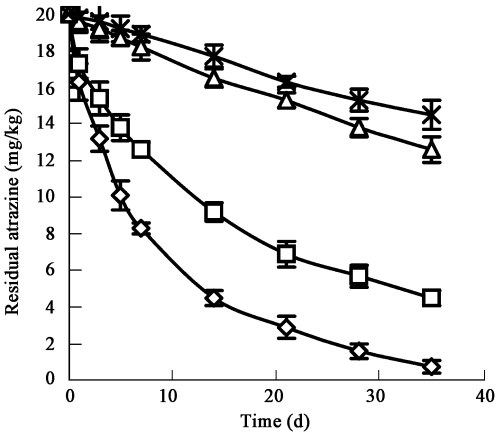

Fig. 1 Degradative kinetic of SIM in soil.

Table 1 Degradative kinetic data of SIM in different soils

Soil	$C = C_0e^{-kt}$	r^2	$t_{1/2}$
Uninoculated non-rhizosphere soil	$C = 20.086e^{-0.0095t}$	0.9974	73.0
Uninoculated rhizosphere soil	$C = 19.944e^{-0.0131t}$	0.9992	52.9
Inoculated non-rhizosphere soil	$C = 17.605e^{-0.041t}$	0.9830	16.9
Inoculated rhizosphere soils	$C = 17.21e^{-0.0883t}$	0.9929	7.8

The degradation rates might be closely related to microbial characteristics. The results of microbial biomass carbon, plate counts of heterotrophic bacteria and most probably the number of SIM degraders in uninoculated non-rhizosphere soil, uninoculated rhizosphere soil, inoculated non-rhizosphere soil, and inoculated rhizosphere soil are shown in Table 2. Microbial biomass carbon was significantly increased in uninoculated rhizosphere soil and inoculated rhizosphere soil, the largest microbial biomass was found in inoculated rhizosphere soil, and the difference of microbial biomass was insignificant between uninoculated rhizosphere soil and inoculated non-rhizosphere soil. The number of culturable heterotrophic microorganisms is similar in uninoculated non-rhizosphere soil and uninoculated rhizosphere soil, but larger numbers of culturable heterotrophic microorganisms were observed in inoculated soil, and numbers of culturable heterotrophic microorganisms in inoculated rhizosphere soil were significantly larger than in inoculated non-rhizosphere soil. Similar results as culturable heterotrophic microorganisms were observed for MPN of SIM degraders in the experiment.

3 Discussion

Accelerated organic contaminant degradation is frequently observed in planted soils but the mechanisms involved are poorly understood. In this study, an attempt is made to correlate SIM degradation patterns with gross measures of community biomass and MPN estimates of SIM degrading microbial populations in grass rhizosphere soils. The primary interest was to determine whether the presence of *Pennisetum* promoted a microbial community capable of degrading SIM, in both inoculated soil and uninoculated soil.

In the present study, whether uninoculated soil or inoculated, higher degradation rates of SIM were observed in rhizosphere soils, especially in inoculated rhizosphere soil, which is consistent with the larger numbers of SIM degraders estimated in the soils rhizosphere soil. It indicated that vegetation had a definitive effect on the degraded fate of SIM in the planted soil compared to unplanted soil. Similar results were also reported by other researchers (Curl and Truelove, 1986; Walton *et al*., 1994; Cunningham *et al*., 1996).

The microbial results in the present study also indicated that there was a close relationship between SIM degradation and microbial communities, microbial biomass, heterotrophic bacterial community. The presence of grasses would enhance the magnitude of rhizosphere microbial communities, total microbial biomass content, and heterotrophic bacterial community, which lead to the shorter half-life of SIM in rhizosphere soil, especially in inoculated rhizosphere soil. The results reconciled other reports of plant-enhanced degradation of organic contaminant (Yu *et al*., 2003; Neer *et al*., 2004). The reasons might be, in the rhizosphere, the immediate vicinity of plant roots, was a zone of intense microbial activity, which was caused by root exudates containing containing carbohydrates, amino acids and organic acids (Curl and Truelove, 1986). Therefore, the use of vegetation at the waste sites could overcome some of the inherent limitations to biological cleanup approach, such as low microbial population or inadequate microbial activity.

By comparing the degradation of SIM and microbial parameters in uninoculated non-rhizosphere soil and uninoculated rhizosphere soil, the difference of degradation rate of SIM, heterotrophic bacterial community and SIM degraders was not significant, which indicated that the presence of grasses would mainly enhance the magnitude of rhizosphere microbial communities, but did

Table 2 Microbial biomass carbon, plate counts of heterotrophic bacteria and most probably number of SIM degraders in different soils

Soil	Biomass carbon (mg/kg)	Culturable heterotrophic bacteria (logCFU/g soil)	MPN of SIM degraders (logMPN cells/g soil)
Uninoculated non-rhizosphere soil	91.4 a*	6.4 a	4.1 a
Uninoculated rhizosphere soil	131.2 b	6.8 a	4.5 a
Inoculated non-rhizosphere soil	124.7 b	7.3 b	6.1 b
Inoculated rhizosphere soil	179.1 c	8.7 c	7.3 c

* Means for a given phase-amendment combination followed by the same letter are not significantly different ($P \leqslant 0.05$).

little to influence those community components responsible comparing for SIM degradation. Similar results were reported by Alvey and Crowley (1996) and Fang *et al*. (2001). However, in inoculated rhizosphere soil, there are the largest numbers of SIM degraders, leading to SIM degradate more rapidly. Therefore, the use of vegetation and inoculating degradation microorganism together might be the best way to overcome some of the inherent limitations to biological cleanup approach, such as low microbial population or inadequate microbial activity for persistent organic contaminant.

4 Conclusions

Vegetation could enhance the magnitude of rhizosphere microbial communities, microbial biomass content, and heterotrophic bacterial community, but did little to influence those community components responsible for comparing SIM degradation. This is most likely the result of C exudation from plant roots into the rhizosphere, which supports an increased microbial population. Use of vegetation and inoculating SIM degradation microorganism could accelerate degradation of SIM in vegetated soil, which suggested that vegetation could also enhance the magnitude of inoculated SIM degrader. Our data clearly demonstrated an rapid degradative rate of SIM in the inoculated rhizosphere soil and as compared to uninoculated non-rhizosphere soil. The results indicated that the inoculation of rhizosphere with microorganisms capable of degrading organic contaminant might be a possible approach to remove or detoxify organic contaminant residues in soil.

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References

Albarrán A, Celis R, Hermosín M C, López-Piñeiro A, Ortega-Calvo J J, Cornejo J, 2003. Effects of solid olive-mill waste addition to soil on sorption, degradation and leaching of the herbicide simazine. *Soil Use and Management*, 19(2): 150–156.

Alvey S, Crowley D E, 1996. Survival and activity of an simazine-mineralizing bacterial consortium in rhizosphere soil. *Environ Sci Technol*, 30: 1596–1603.

Cunningham S D, Anderson T A, Schwab A P, Hsu F C, 1996. Phytoremediation of soil contaminated with organic pollutant. *Adv Agron*, 56: 55–114.

Curl E A, Truelove B, 1986. The Rhizosphere. Berlin: Springer.

Damaj M, Ahmad D, 1996. Biodegradation of polychlorinated biphenyls by rhizobia, a novel finding. *Biochem Biophys Res Commun*, 218: 908–915.

Dandurand L M, Schotzko D J, Knudsen G R, 1997. Spatial patterns of rhizoplane populations of *Pseudomonas fluorescens*. *Appl Environ Microbiol*, 63: 3211–3217.

Fang C W, Radosevich M, Fuhrmann J J, 2001. Atrazine and phenanthrene degradation in grass rhizosphere soil. *Soil Biol Biochem*, 33: 671–678.

Holden P A, Firestone M K, 1997. Soil microorganisms in soil cleanup: How can we improve our understanding? *J Environ Qual*, 26: 32–40.

Joergensen R G, 2000. Ergosterol and microbial biomass in the rhizosphere of grassland soils. *Soil Biol Biochem*, 32: 647–652.

Molina L, Ramos C, Duque E, Ronchel M C, Ronchel M C, Garcia J M, Wyke L, Ramos J L, 2000. Survival of *Pseudomonas putida* KT2440 in soil and in the rhizosphere of plants under greenhouse and environmental conditions. *Soil Biol Biochem*, 32: 316–321.

Neera S, Megharaj M, Kookana R S, Naidu R, Sethunathan N, 2004. Atrazine and simazine degradation in Pennisetum rhizosphere. *Chemosphere*, 56: 257–263.

Nicholes T D, Wolf D C, Rogers H B, Beyrouty C A, Reynolds C M, 1997. Rhizosphere microbial populations in contaminated soils. *Water Air Soil Pollut*, 95: 165–178.

Reilley K A, Banks M K, Schwab A P, 1996. Dissipation of polycyclic aromatic hydrocarbons in the rhizosphere. *J Environ Qual*, 25: 212–219.

Spliid N H, Kopper B, 1998. Occurrence of pesticides in Danish Shallow ground water. *Chemosphere*, 37: 1307–1316.

Steer J, Harris J A, 2000. Shifts in the microbial community in rhizosphere and non-rhizosphere soils during the growth of *Agrostis stolonifera*. *Soil Biol Biochem*, 32: 869–878.

Vance E D, Brookes P C, Jenkinson D S, 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol Biochem*, 19: 703–707.

Walton B T, Guthrie E A, Hoylmann A M, 1994. Toxicant degradation in the rhizosphere. In: *Bioremediation through Rhizosphere Technology* (Anderson T. A., Coats J. R., eds.). Washington: American Chemical Society: 11–26.

Wehtje G R, Spading R F, Burnside O C, Lowry S R, Leavitt J C R, 1983. Biological significance and fate of atrazine under acquifer conditions. *Weed Sci*, 31: 610–618.

Yu Y L, Chen Y X, Luo Y M, Pan X D, He Y F, Wong M H, 2003. Rapid degradation of butachlor in wheat rhizosphere soil. *Chemosphere*, 50: 771–774.