Degradation kinetics and products of triazophos in intertidal sediment

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Abstract: This work presents laboratory studies on the degradation of triazophos in intertidal sediment. The overall degradations were found to follow the first-order decay model. After being incubated for 6 d, the percentage of degradations of triazophos in unsterilized and sterilized sediments were 94.5% and 20.5%, respectively. Between the temperatures of 15℃ and 35℃, the observed degradation rate constant(K_{obsd}) enhanced as the incubation temperature increased. Triazophos in sediment degraded faster under aerobic condition than under anaerobic one. The water content of sediment had little influence on the degradation when it was in the range of 50%—100%. The values of K_{obsd} decreased with increasing initial concentration of triazophos in sediment, which could result from the microorganism inhibition by triazophos. Four major degradation products, o, o-diethyl phosphorothioic acid, monoethyl phosphorothioic acid, phosphorothioic acid, and 1-phenyl-3-hydroxy-1, 2, 4-triazole, were tentatively identified as their corresponding trimethylsilyl derivatives with a gas chromatography-mass spectrometer. The possible degradation pathway of triazophos in intertidal sediment was proposed. The results revealed that triazophos in intertidal sediment was relatively unstable and could be easily degraded.

Keywords: triazophos; degradation; intertidal sediment

Introduction

Triazophos(CAS Registry 24017-47-8) with a chemical name as o, o-diethyl o-1-phenyl-1H-1, 2, 4-triazol-3-yl phosphorothioate, is a moderately toxic and broad-spectrum, nonsystemic organophosphorus pesticide. It has been put into agricultural use since the late 1970s, on various crops such as cotton, maize, paddy, and vegetable. Triazophos is highly toxic to most fishes, but lowly toxic to shellfishes. In recent years, it has been also utilized as fungicide in intertidal aquiculture to protect farming shellfish such as *Sinonovacula constricta* and *Tegillarca granosa* from diseases. Due to the misuse of triazophos in intertidal aquiculture, fish kill accidences caused by triazophos pollution took place sporadically in Fujian and Zhejiang coastal areas in China. The evaluation of environmental safety for triazophos used in intertidal aquiculture is thus of great concern.

Triazophos applied on intertidal sediment may subject to abiotic and biotic degradations. The major abiotic transformations include hydrolysis, photodegradation, and redox reaction. Although the disappearances of triazophos in water or soil have been investigated (Lin, 2004a; 2004b; Sunita, 2001; Bock, 1975), little is known about its degradation in intertidal sediment, especially its kinetics and degradation products. The objectives of this work were to explore triazophos persistence in intertidal sediment under laboratory conditions and to provide a better base, on which to evaluate environmental safety of triazophos in intertidal aquiculture use.

1 Materials and methods

1.1 Instrumentals

The analysis of triazophos residue extracted from the tested intertidal sediment was performed by a gas chromatography (GC) with a flame photometric detector (FPD) operated at phosphorus mode (Agilent Technologies Inc., Palo Alto, CA) with the following parameters: injection port, 250℃; splitless injection; 1 μl injection volume; capillary column, SPB1701 (Supelco, Bellefonte, PA), 30 m × 0.32 mm i.d. and 0.25 μm film thickness; column temperature program, initial temp. 160℃, 20℃/min

to 260℃(6 min); carrier gas nitrogen 1.5 ml/min, make up gas nitrogen 25 ml/min, air 100 ml/min, hydrogen 75 ml/min; detector, 250℃. The retention times of triazophos and surrogate triphenyl phosphate were 8.91 and 9.13 min, respectively.

The identification of derivatives of triazophos degradation products was performed on a Varian 3900 GC directly connected to a Saturn 2000 ion trap mass spectrometer (Varian, Palo Alto, CA). The mass spectrometer operated at an electron impact auto mode with 70 eV of ionization energy was used for full scan in a m/z range from 60 to 500. Other parameters were as follows: standard injection port, 250℃; splitless injection; 1 μl injection volume; capillary column, VF-5 MS(Varian, Palo Alto, CA), 30 m × 0.25 mm i.d. and 0.25 μm film thickness; column temperature program, 80℃(3 min) – 10℃/min – 280℃(7 min); carrier gas flow rate, helium 1.0 ml/min; manifold, trap, and transfer line temperatures were set at 40, 180, and 280℃, respectively.

1.2 Chemicals and reagents

Triazophos with a purity higher than 97.5% was purchased from Kefa New Technology Development Ltd Co. (Shenyang, China). High-performance liquid chromatography grade acetone, ethyl acetate and hexane were purchased from Tedia Company (Fairfield, OH). Triphenyl phosphate with a purity higher than 99% was purchased from Acros Organics (Geel, Belgium). The derivatization reagent n, o-bis (trimethylsilyl) trifluoroacetamide (BSTFA) containing 1 vol % by volume trimethylchlorosilane (TMCS) was purchased from Supelco Inc. (Bellefonte, PA). Analytical reagents anhydrous sodium sulfate and sulfuric acid were purchased from Shanghai Chemical Regents Co. (Shanghai, China).

1.3 Sediment and seawater

An intertidal sediment sample was collected from intertidal mudflat at Zhao'an Bay, Fujian, China(23°48′25.2″N, 117°32′22.3″E). The sediment sample was taken back to the laboratory and air-dried naturally under cool condition, then passed through a 2 mm sieve before use. The prepared sediment was stored in closed plastic bags at room temperature and used within one month after sampling. The sample was a sandy loam with 81.1% sand, 10.5% silt,

7.0% clay, 0.64% organic matter, and with pH 8.55. The sediment pH was measured in a 1:5(m:v) sediment:water suspension using a glass electrode; organic substance content by the Walkley and Black method (Walkley, 1934) and sediment mechanical fractions by the hygrometer method (Black, 1982). The natural seawater with pH 8.13 and salinity 24.5‰ was filtered through 0.45 μm membrane before use. Both the sediment and seawater samples contained no detectable amount of triazophos and its degradation products. To compare biotic and abiotic degradation, a portion of both sediment and seawater sample was autoclaved at 121℃ for 60 min to remove microbial activities.

1.4 Preparation of solutions

A triazophos stock solution of 5000 μg/ml and a triphenyl phosphate of 10 μg/ml were prepared by dissolving the analyte in acetone, respectively. Working standards were prepared by diluting the stock solutions with acetone. All the solutions were stored in a freezer at -4℃ while not in use.

1.5 Degradation experiment

Aliquots of 10.0 g dry weight of sediment, in duplicate, were placed in 100 ml flasks and spiked with a certain amount of triazophos standard solution. Gentle air streams were passed through the flasks to evaporate the acetone, then certain volumes of seawater were added into and mixed with the sediments thoroughly. All the prepared samples containing designed amount of seawater and triazophos were incubated in the dark to process the degradation. For aerobic degradation experiments, the flasks were opened to the air. In the case of anaerobic studies, the flasks were sealed with rubber plugs immediately after being filled with nitrogen. The aerobic incubation samples were weighed periodically to check water loss, and sterilized deionized water was added to compensate the water loss during the incubation. Duplicate of samples were taken for analyzing triazophos residue at intervals ranging from 1 to 2 d, depending on the preliminary observations of degradation rates.

1.6 Determination of triazophos residue in sediment

A method for the determination of triazophos residue in the sediment was developed (Lin, 2004c). The analysis procedures were briefly described as follows. One hundred microliters of triphenyl phosphate of 10 μg/ml were added in each sediment as surrogate before the extraction for data quality control. Certain amount of deionized water was added into the sediment to make its water content about 100% (v/w), then the sample was extracted 3 times, each with 15 ml of hexane and magnetic stirring for 10 min. The organic phases were collected and combined together. The extract was filtered with a glass funnel filled with anhydrous sodium sulfate and then evaporated to nearly dryness with a gentle nitrogen stream in a 40℃ water bath. The final volume 1.0 ml was made with hexane, and the extract sample was ready for GC analysis.

The recoveries of triazophos with the concentration of 0.1 μg/g were 87%—95%, and the relative standard deviation was 4.3% (n = 3). Linearity was between 0.01—1.0 μg/g with the correlation coefficient of 0.9994(n = 6). The method detection limit of triazophos was 0.003 μg/g. Recoveries of triphenyl phosphate was within 88%—93%.

1.7 Identification of degradation products

Degradation product identification was carried out at the end of one half-life. One hundred grams of incubation sediment were withdrawn for extracting the degradation products. The extraction procedures involved two steps: (1) the tested sample was extracted twice each with 150 ml of hexane to get the hydrophobic products. The organic phases were collected while the sediment was taken for further treatment to extract possible hydrophilic compounds; (2) the residual sediment was washed with 150 ml of deionized water and stirred for 15 min. Then the sample was centrifuged at 3500 r/min and the clear supernatant liquid was decanted carefully. The liquid was acidified to pH 2 with 10% (v/v) H_2SO_4 and then freeze-dried to dryness, and the residue was dissolved with 50 ml ethyl acetate. Extracts of step (1) and (2) were combined together and the moisture in them was removed by anhydrous sodium sulfate. The combined extract was concentrated to 0.5 ml with a gentle nitrogen stream and in a 40℃ water bath. To silylate the extracted compounds, the extract and silylating reagent BSTFA-1% TMCS, 100 μl each, were well mixed in a septum-capped glass vial and allowed to react for 2 h in a 60℃ water bath. The large excess of BSTFA-1% TMCS ensured a complete derivatization. An aliquot of 1 μl silylated extract sample was taken for GC-MS analysis.

2 Results and discussion

2.1 Effect of microorganisms

The degradation of triazophos in the intertidal sediment mainly involves biotic and abiotic processes. To compare biotic and abiotic degradations, decompositions of triazophos in unsterilized and sterilized sediments were performed and the result is shown in Fig. 1. It can be seen that the percentage of triazophos degraded in the unsterilized sediment was higher than that in the sterilized sediment as the incubation proceeded. After being incubated for 6 d, the percentage of triazophos degradation in the unsterilized sediment was 94.5%, while only 20.5% in the sterilized one. Therefore, it can be inferred that microorganisms play a vital role in the triazophos transformation in the intertidal environment. To make the laboratory experiments imitate the degradation of triazophos in real intertidal sediment, the following experiments were carried out with unsterilized sediment.
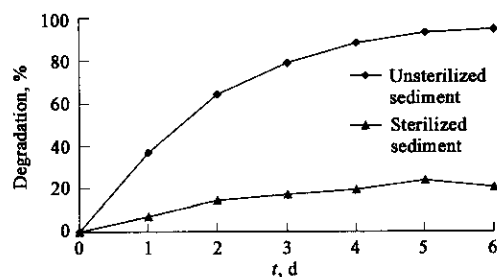

Fig.1 Comparative degradations of triazophos in unsterilized and sterilized sediments
Conditions: aerobic incubation temperature, 35℃; initial triazophos concentration, 0.500 mg/kg; water content of sediment, 75%

2.2 Effect of incubation temperature, aerobic/anaerobic conditions, and water content of sediment

Degradations of triazophos in the intertidal sediment under different conditions were investigated. Although the degradation of triazophos in the intertidal sediment involved both biotic and abiotic processes, it was found that the overall decompositions of triazophos in the sediment under different conditions could be treated as first-order reaction.

Thus, the following equations might be applied

$$\mathrm{d}C/\mathrm{d}t = -k_{obsd}C, \tag{1}$$

or

$$\ln(C/C_0) = -k_{obsd}t, \tag{2}$$

where C_0 and C are the triazophos concentrations in the initial sediments and the samples collected at a designed reaction time, respectively; and k_{obsd} is the observed rate constant for triazophos degradation. When the plot of $\ln(C/C_0)$ versus reaction time is derived, k_{obsd} is equal to the slope. The kinetic data of triazophos degradation in intertidal sediment under different conditions are listed in Table 1. It shows that the degradation fit very well with first-order reaction model, with r values ranging between 0.9834 and 0.9972.

Table 1 Kinetic data of triazophos degradation in intertidal sediment under different conditions[a]

T, ℃	Aerobic or anaerobic	Water content[b], %	k_{obsd}, d^{-1}	$t_{1/2}^{c}$, d	$r(n=7)$
15	Aerobic	75	0.0891	7.78	0.9967
25	Aerobic	75	0.280	2.48	0.9927
35	Aerobic	75	0.508	1.36	0.9972
35	Anaerobic	75	0.340	2.04	0.9834
35	Aerobic	50	0.453	1.53	0.9950
35	Aerobic	100	0.530	1.31	0.9935

Notes: [a] Initial concentration of triazophos in sediments, 0.500 mg/kg; [b] water content(%) $= \dfrac{\text{weight of water}}{\text{dry weight of sediment}} \times 100\%$; [c] half-life was calculated as $t_{1/2} = (\ln 2)/k_{obsd}$

The $t_{1/2}$ of triazophos in sediment at 15℃ was 7.78 d, and it decreased to 1.36 d at 35℃. Within the temperatures of 15—35℃, a larger k_{obsd} could be obtained under higher incubation temperature(Table 1). There might be two reasons for this. One is that the chemical degradation was accelerated by higher temperature, and the other is that microbial activities were enhanced with increasing temperature, resulting in a faster biotic transformation. This result was well consistent with the temperature effect on degradations of some other pesticides in soils(Neera, 2005; Sarah, 2002). It can be deduced that the degradation of triazophos in real intertidal sediment is faster in summer than that in winter.

To study the influence of aerobic and anaerobic conditions on the biodegradation, the values of k_{obsd} under aerobic and anaerobic incubation were determined. The result in Table 1 shows that triazophos decomposed faster under aerobic condition($t_{1/2} = 1.36$ d) than that under anaerobic ones($t_{1/2} = 2.04$ d). However, it could not be inferred that the aerobes played more important role than the anaerobes did, because there might be facultative microbes that could transform triazophos under either condition.

The values of k_{obsd} determined from sediments with different water contents varied a little(Table 1). The $t_{1/2}$ at water content of 50% was 1.53 d, and at 100% was 1.31 d. Sediment with water content of 50% could nearly imitate the moisture of real intertidal sediment at low tide, while 100% at high tide. Therefore, it could be included that triazophos in real intertidal sediment degrades somewhat faster in sediment with higher water content.

2.3 Effect of initial concentration of triazophos

To study the influence of triazophos initial concentration on degradation, the values of k_{obsd} were determined with the initial concentrations of triazophos ranging from 0.500 mg/kg to 10.0 mg/kg, and the kinetic data are listed in Table 2. The results showed that the triazophos degradations at various initial concentrations fit very well with the first-order reaction

model, with an r range within 0.9790—0.9972. It can be also seen from Table 2 that the values of k_{obsd} decreased as the initial triazophos concentration increased. It seems that the result was paradoxical to the principle of first-order reaction, because the initial concentration of substrate does not influence k_{obsd} for a pure chemical reaction that follows first-order reaction model. In fact, the overall degradation rate constant k_{obsd} is the sum of chemical degradation rate constant k_c and microbial degradation rate constant k_b(Qin, 2004), i.e.

$$k_{obsd} = k_b + k_c. \tag{3}$$

The comparison of triazophos degradation in sterilized and unsterilized sediments had suggested that the k_{obsd} might be primarily attributable to microbial transformation that depended on the microorganism activities. Triazophos is, however, a kind of pesticide and can inhibit or even kill microorganisms in the sediment. The decreased of k_{obsd} could be due to the enhancing inhibition effect as the initial concentration of triazophos in the sediment increased.

Table 2 Kinetic data of triazophos degradation in intertidal sediment with various initial triazophos concentrations[a]

C_0, mg/kg	k_{obsd}, d^{-1}	$t_{1/2}$, d	$r(n=7)$
0.500	0.508	1.36	0.9972
1.000	0.455	1.52	0.9941
2.000	0.372	1.86	0.9948
5.000	0.328	2.11	0.9942
10.00	0.262	2.65	0.9790

Notes: [a] Conditions: aerobic incubation temperature, 35 ℃; water content of sediment, 75%
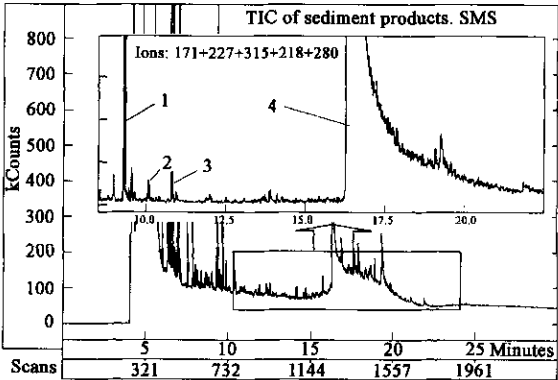

Fig.2   TIC chromatogram in the full-scan mode for trimethylsilyl derivatives of triazophos degradation products in intertidal sediment
Conditions: aerobic incubation temperature, 35℃; initial triazophos concentration, 2.000 mg/kg; water content of sediment, 75%

2.4 Degradation products and proposed pathway

The triazophos degradation products were investigated when the reaction was at the end of a half-life(45 h). The total ion current(TIC) chromatogram of GC-MS is presented in Fig.2 and the mass spectra of peaks 1—4 are shown in Fig.3—6. Through the interpretation of mass spectra, peaks 1—4 in Fig. 2 could be tentatively identified as the corresponding trimethylsilyl derivatives of o, o-diethyl phosphorothioic acid (P-I), monoethyl phosphorothioic acid (P-II), phosphorothioic acid (P-III), and 1-phenyl-3-hydroxy-1,2,4-triazole(P-IV), respectively. All of the four degradation products were also detected in the hydrolysis of triazophos(Lin, 2004b). From the physicochemical point of view, it can be predicted that the toxicities of the detected degradation products are lower than triazophos. On the basis of products detected, a possible degradation pathway of

triazophos in intertidal sediment is proposed in Fig. 7. Although phenylsemicarbazine (P-V) was also identified as the degradation product of triazophos in soil(Bock, 1975), it was not detected in this study. The reason may be that the

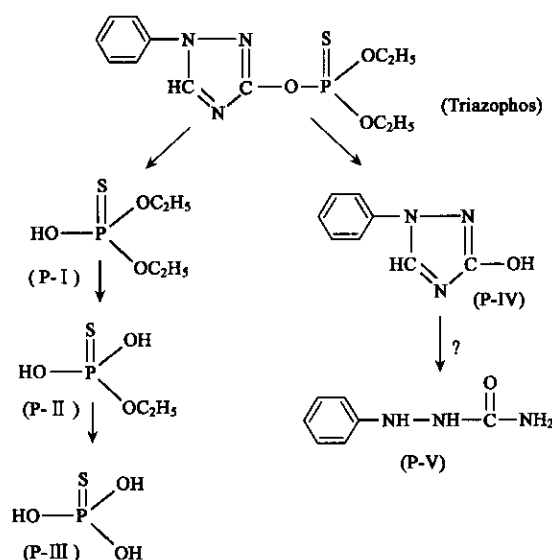breakdown of P-IV to P-V was relatively slow and P-V could not be formed in a half-life degradation.
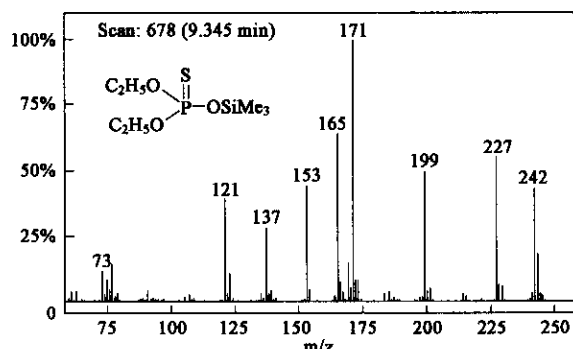

Fig. 3   Mass spectrum of peak 1 and its corresponding compound
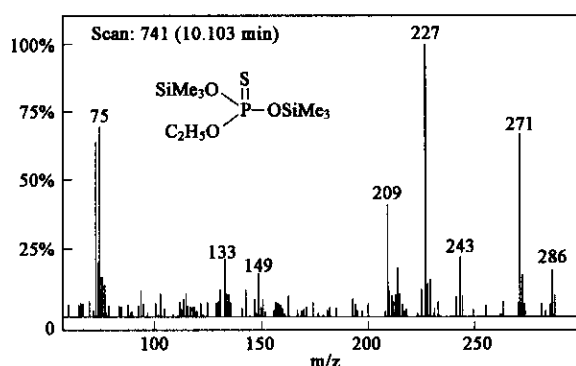

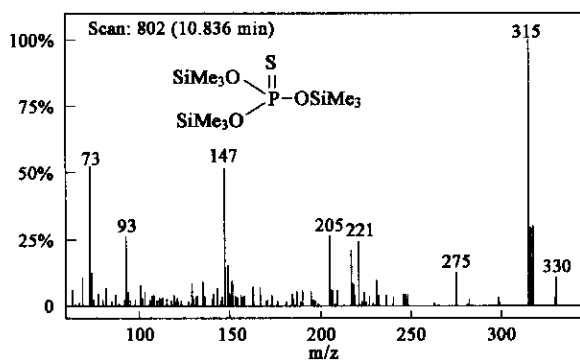Fig. 4   Mass spectrum of peak 2 and its corresponding compound

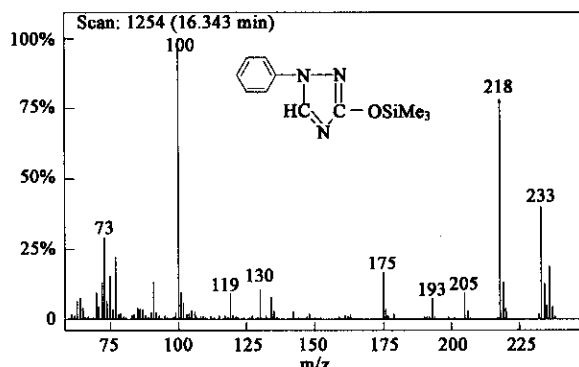Fig. 5   Mass spectrum of peak 3 and its corresponding compound

Fig. 6   Mass spectrum of peak 4 and its corresponding compound

Fig. 7   Possible pathways for the degradation of triazophos in intertidal sediment

3   Conclusions

This study indicated that triazophos in intertidal sediment was relatively unstable and could be easily broken down. Microorganisms played a vital role in triazophos degradation. Triazophos in intertidal sediment could be degraded faster in higher temperature environment or under aerobic condition. High concentration of triazophos in sediment can inhibit the degradation.

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