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Occurrence and dissipation of three azole biocides climbazole, clotrimazole and miconazole in biosolid-amended soils

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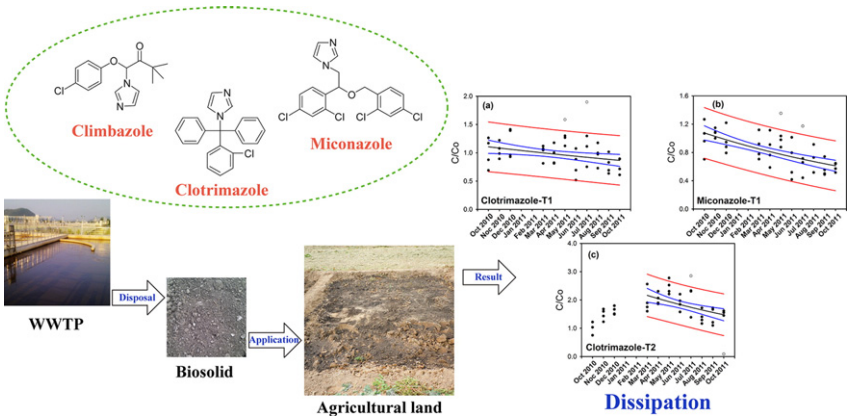
HIGHLIGHTS

- Climbazole, clotrimazole and miconazole persisted in the biosolid-amended soils.
- Three azole biocides are more readily dissipated under the flooding condition.
- Climbazole was more persistent than the other two compounds in the soils.

GRAPHICAL ABSTRACT



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ABSTRACT

This study investigated the occurrence and dissipation of three azole biocides climbazole, clotrimazole and miconazole in biosolid-amended soils of the three sites (Zhejiang, Hunan and Shandong) in China following three treatments (CK: control without biosolid application; T1: one biosolid application; T2: biosolid application every year). The results showed that climbazole, clotrimazole and miconazole were present in the biosolid and biosolid-amended soils, but absent in the control soils. In the soils treated with biosolids, the concentrations of climbazole, clotrimazole and miconazole were mostly lower in the Zhejiang soils than in the Shandong or Hunan soils, suggesting that these three biocides are more readily dissipated under the flooding condition. During the one year monitoring, the concentrations of climbazole, clotrimazole and miconazole in the biosolid-applied soils showed only slight variations. The dissipation half-lives for miconazole calculated under the field conditions of Shandong site were 440 days for T1 and the half-lives for clotrimazole were 365 days for T2. The results suggested the persistence of these three biocides in the soil environments.

1. Introduction

Azole biocides such as climbazole, clotrimazole and miconazole are widely used as antifungal agent in pharmaceutical and personal care products. The mode of action is their blocking of sterol synthesis

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by inhibiting cytochrome P450-dependent 14α-demethylase (Zarn et al., 2003). After application, these azoles can be removed from the body by washing or removed through urinary excretion, and enter wastewater treatment plants (WWTPs). Due to incomplete removal of azoles during wastewater treatment, they finally reach the receiving environment via discharge of effluent and disposal of sewage sludge (Peng et al., 2012).

Azole biocides have been reported in effluent, surface water, and sludge (Huang et al., 2010; Wick et al., 2010). For example, climbazole has been detected with concentrations ranging between 312 and 443 ng/L in WWTP effluents, 47 and 530 ng/L in the tributaries of the Main River, and 1160 ng/g in activated sludge in Germany (Wick et al., 2010). Clotrimazole and miconazole have been detected with the concentrations of 8 and 3 ng/L in effluent, 4 and 2 ng/L in the Pearl River, and 190–1442 ng/g and 238–1405 ng/g in sewage sludge in southern China (Huang et al., 2010). It has become a concern that the presence of these azole biocides in the environment may cause adverse effects in nontarget organisms. In addition to the inhibition of cytochrome P450 enzymes, azoles have been shown to have endocrine disrupting activities (Kjaerstad et al., 2010). For example, clotrimazole has been found to stimulate the ligand-binding domain (LBD) of mammalian and zebrafish PXR genes (Moore et al., 2002). The azoles such as clotrimazole and miconazole could affect the aromatase enzyme in rainbow trout, frogs and human (Gyllenhammar et al., 2009; Monod et al., 1993; Trosken et al., 2004).

One of the entry pathways to the environment for these azole biocides is via the application of sewage sludge as fertilizer (biosolid) in agriculture, which has been considered as a sustainable practice in many countries such as Australia, United Kingdom and United States (Duarte-Davidson and Jones, 1996; Langdon et al., 2012; USEPA, 2012). In China, the application of biosolids on agricultural land is still not allowed due to concerns with various contaminants in the biosolid (Daughton and Ternes, 1999; Gottschall et al., 2012; Kinney et al., 2008; Langdon et al., 2012; McClellan and Halden, 2010; Walters et al., 2010). Chinese Ministry of Agriculture initiated field trials of biosolids in 2006 to address the concerns associated with biosolid application on agricultural land. As part of the program, the fate of organic contaminants including azole biocides in biosolid-amended soils has been investigated in addition to inorganic contaminants (Li et al., 2012). Limited previous studies showed the persistence of the azole biocides such as clotrimazole in soils under laboratory conditions (Garcia-Valcarcel and Tadeo, 2012; Sabourin et al., 2011). Dissipation behavior of organic contaminants under field conditions can be very different to that under laboratory conditions (Langdon et al., 2012). However, the study about dissipation of these azole biocides under field conditions is still very limited (Gottschall et al., 2012).

The aims of this study were to investigate the occurrence and fate of the three common azole biocides climbazole, clotrimazole and miconazole in biosolid-amended soils under field conditions. Three sites with different field conditions from Zhejiang, Hunan and Shandong, China were selected to carry out the field trials. The results from this study can help assess potential impacts of these biocides on the environment.

2. Materials and methods

2.1. Chemicals and materials

The high purity standards of climbazole (99%) and clotrimazole (99%) were purchased from Dr. Ehrenstorfer GmbH (Germany), while miconazole (approximately 100%) was obtained from the United States Pharmacopeia (USA). Isotope-labeled internal standards clotrimazole-D5 and imazalil-D5 were supplied by Toronto Research Chemicals (Canada) and Dr. Ehrenstorfer GmbH (Germany), respectively. The physicochemical properties of these three azole biocides are given in Table 1. All the organic solvents were HPLC grade and purchased from Merck Corporation (China), CNW Technologies (Germany) and Teida Company (USA). Deionized water was obtained from a Milli-Q water purification system (Millipore, Watford). Oasis HLB cartridges (200 mg, 6 mL) were supplied by Water Corporation (USA). Individual stock solutions of the target analytes and internal standards were prepared at 100 mg/L in methanol and stored in amber glass bottles at −18 °C. Then the working solutions were made by appropriate dilution as required.

2.2. Field trials

Field trials of biosolid application on agricultural land were carried out at three sites (ZJ: Zhejiang, HN: Hunan and SD: Shandong) in China. Three treatments included in the trials were: the controls without the application of biosolid (CK), one biosolid application (T1) and repeated application every year (T2). The biosolid which was used for application at the three sites was dewatered sludge from a wastewater treatment plant (WWTP) in Beijing and collected in May 2006. Meanwhile, the dried biosolid was stockpiled in a warehouse before use and these biosolids were always applied in each treatment mentioned in this study. Biosolid samples were collected every year and stored in a fridge for chemical analysis. Each treatment had four replicate plots (3 × 2 m, each). The biosolid was first applied at the three sites on the 31st May 2007 at a rate of 60 t/ha. For T1, only one application was carried out, while for T2, repeat applications were carried out on the 5th October every year following the first application till October 2010. In each treated plot, the biosolids were spread randomly over the fields and mixed well using hoe with the soil of 0–20 cm depth immediately following application.

Although the field trials started in May 2007, sampling campaign for organic contaminants was only carried out from the beginning of October 2010 to October 2011. Initial field trials focused on inorganic contaminants in the biosolid amended soils (Li et al., 2012). Soil samples were collected in 1 L glass jars from each field plot at the depth of 0–20 cm from five points in each plot and then combined into one composite sample. The first sampling was taken place in the three trial sites on the 5th of October in 2010 prior to the re-application of biosolid. After the first sampling, Zhejiang and Hunan trial sites were closed down due to the logistical problem in transport of the biosolid since the two sites were far away from Beijing. Moreover, the soil samples from the Shandong site were sampled subsequently on the 5th of every month until October 2011. However, due to the frost period in the Shandong site, no soil samples were collected in January and February 2011. The collected soil samples and biosolid samples were freeze-dried, then sieved through a 0.90 mm mesh standard screen and then stored in the dark at 4 °C prior to extraction.

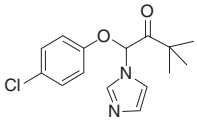
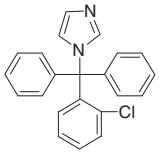
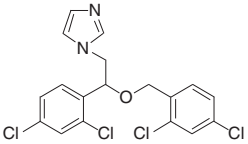
Table 2 shows the information about the three field trial sites. The crops in the three sites were: rice and rape in the Zhejiang site, wheat and maize in both the Hunan and Shandong sites. The annual temperature was 15.9, 19.1 and 12.9 °C for the Zhejiang, Hunan and Shandong sites; while the annual rainfall was 1168, 1360 and 522 mm, respectively. Soil pH was determined with 0.01 M $CaCl_2$ (soil to solution ratio of 1:5) using a pH meter; total organic carbon content (TOC) of soil was measured by a LECO carbon and nitrogen analyzer; and soil particle size distribution was analyzed by using the pipette method (Schinner et al., 1999).

2.3. Chemical analysis

2.3.1. Ultrasonic extraction

The analytical method was developed by modifying our previous method for extracting the target biocides in solid samples by ultrasonic extraction (Chen et al., 2012). Each lyophilized and homogenized solid sample (2 g for soil and 0.5 g for sludge sample) was weighted into a 30 mL glass tube, followed by the addition of 100 μL of 1 mg/L mixed

Table 1
Physicochemical properties of the three biocides investigated in this study.

Compound	Climbazole	Clotrimazole	Miconazole
Structure			
Formula	$C_{15}H_{17}ClN_2$	$C_{22}H_{17}ClN_2$	$C_{18}H_{14}Cl_4N_2O$
CAS number	38083-17-9	23593-75-1	22916-47-8
Molecular weight	292.8	344.8	416.1
Water solubility (mg/L at 25 °C)	8.281[a]	0.03[a]	0.011[a]
Log K_{ow}	3.76[a]	4.1/6.26[b]	6.25[a]
pKa	7.5[c]	6.12[b]	6.65[d]
Log K_{oc}	3.655[a]	4.786[a]	4.834[a]

[a] The log K_{ow} values were calculated by EPI suite model (USEPA, 2012).
[b] Data from OSPAR (2005).
[c] Calculated by ALOGPS 2.1 (Tetko et al., 2005).
[d] Data from Vanden et al. (1987).

internal standard solutions (clotrimazole-D5 and imazalil-D5). Then the samples were mixed and stored in a cold room (4 °C) overnight. Ten milliliters of methanol was added to each sample. The sample was then mixed by a vortex mixer for 30 s, extracted in an ultrasonic bath for 15 min and centrifuged at 2800 rpm for 10 min. The clear supernatant was collected into a 250 mL flat-bottomed flask by a glass pipette. The extraction procedure was repeated twice using 10 mL of methanol and then 10 mL of methanol/0.1% (v/v) formic acid in Milli-Q water (5:5, v/v) as the extraction solvent, respectively. The supernatants were combined and diluted with Milli-Q water to a volume of 300 mL.

2.3.2. Clean-up using solid phase extraction

Oasis HLB cartridges (200 mg, 6 mL) were applied for the cleanup of the aqueous solid extracts. Prior to the SPE cleanup, 4 M H_2SO_4 was used to adjust the pH value of the aqueous extracts to 3. Each cartridge was preconditioned consecutively with 10 mL of methanol and 10 mL of Milli-Q water before use. The aqueous extract was loaded onto the SPE cartridge at a flow rate of 5–10 mL/min. Each sample bottle was rinsed twice with two aliquots of 50 mL of 5% (v/v) methanol in Milli-Q water. These aliquots were also passed through the cartridge after sample loading. The cartridge was dried under vacuum for 3 h, and the target compounds were eluted with 3 × 4 mL of ethyl acetate. The eluate was dried under a gentle nitrogen stream, redissolved in 1 mL of methanol, then filtered through a 0.22 μm membrane filter (Anple, Shanghai, China) into a 2 mL amber glass vial (Agilent, USA) and stored at −18 °C until high performance liquid chromatography coupled to mass spectrometry (LC–MS/MS) analysis. Prior to the LC–MS/MS analysis, 100 μL aliquot of each sample extract solution was dried and reconstituted in a mixed solvent (50% methanol in Milli-Q water, v/v).

2.3.3. LC–MS/MS

The instrumental analysis for the three target compounds also followed our previous method (Chen et al., 2012). The target compounds were determined using an Agilent 1200 series high performance liquid chromatography (Agilent, USA) coupled to an Agilent 6460 triple quadrupole mass spectrometry with electrospray ionization (ESI) in positive ionization mode (LC–MS/MS). The chromatographic column was a Zorbax SB-C18 (100 mm × 3 mm, 1.8 μm particle size) column with its corresponding pre-column filter (2.1 mm, 0.2 μm) from Agilent Technologies for chromatographic separation of these target compounds. Detailed information about LC operating parameters can be found in the Supplementary Information (SI) (Table S1).

The mass spectrometry parameters including fragmentor voltage, collision energy (CE), precursor ion and product ions for each compound, were optimized by Optimizer (Agilent, USA). More detailed mass spectrometric operating parameters and source parameters can be referred to Tables S1–S2. The quantification of the target compounds was performed in multiple reaction monitoring (MRM) mode. Data acquisition was performed by Agilent Mass Hunter B 03.01 software, while the identification of the target compounds was based on their retention times (within 2%) and the ratios of the two selected precursor–product ion transitions (within 20%) in comparison with the corresponding standards.

Table 2
Information of the field trial sites and treatments.

Treatment [a]	Location	Crops	Annual average temperature (°C)	Annual total rainfall (mm)	Soil type/texture	Soil moisture (%)	pH[b]	TOC (%) [c]	Clay (<0.002 mm) (%)	Biosolid application (t/ha)
ZJ-CK	Zhejiang (ZJ)	Rice and rape	15.9	1168	Paddy soil/silt loam	100	6.3 ± 0.5	1.4 ± 0.4	9.7 ± 9.2	0
ZJ-T1							6.8 ± 0.2	2.1 ± 0.6	11.2 ± 6.8	60 once
ZJ-T2							7.1 ± 0.1	1.2 ± 0.2	9.7 ± 1.0	60 every year
HN-CK	Hunan (HN)	Wheat and maize	19.1	1360	Red soil/loam	24–25	4.3 ± 0.1	1.0 ± 0.1	10.3 ± 1.7	0
HN-T1							5.6 ± 0.1	1.3 ± 0.1	9.6 ± 1.0	60 once
HN-T2							7.0 ± 0.2	2.4 ± 0.2	7.4 ± 3.5	60 every year
SD-CK	Shandong (SD)	Wheat and maize	12.9	522	Fluvo-aquic soil/clay loam	23	7.6 ± 0.2	0.6 ± 0.0	21.7 ± 4.2	0
SD-T1							7.6 ± 0.1	1.0 ± 0.1	21.9 ± 1.5	60 once
SD-T2							7.5 ± 0.1	1.4 ± 0.3	26.0 ± 0.8	60 every year

[a] The treatments at each site include control (CK), treatment 1 (T1), and treatment 2 (T2);
[b] Mean ± standard deviation (%) (n = 3). All the pH, TOC and clay content values were detected in the samples collected in October 2010;
[c] TOC, the total organic carbon content.

2.4. Validation of extraction procedure

Recovery tests of the target compounds were carried out by spiking three known concentrations of the target standards (40, 100 and 200 ng/g for sludges and 20, 50 and 100 ng/g for soils) into sludge and soil samples in three replicates. The limits of detection (LOD) and limits of quantitation (LOQ) were defined as three and ten times of signal-to-noise (S/N) ratio under the lowest spiked concentration in those sludge and soil samples. The analytical method for the three biocides showed satisfactory performance with their recoveries of 79.9 to 102% and 73.4 to 103% from the sludges and soils, as shown in Table S3. The LOQs of climbazole, clotrimazole and miconazole were 0.13, 0.14 and 0.38 ng/g for the sludge samples and 0.02, 0.02 and 0.02 ng/g for the soil samples, respectively (Table S3).

All data obtained from the analysis were subject to strict quality control procedures. For each batch of samples to be analyzed, a solvent blank, a standard solution (100 μg/L) and a method blank were run in sequence to check for background contamination and instrument performance.

2.5. Data analysis

A one-way ANOVA and paired samples statistics were performed to determine significant differences ($p < 0.05$) between the concentration data of the three biocides in the three different type soils and different treatments. Prior to all nonlinear regression fitting, the concentration data from the Shandong site were converted to a ratio of the initial concentration (C/C_o). C_o represented the average of the initial value. Nonlinear regressions were used to determine the dissipation patterns of each compound. A standard first-order exponential decay model with two fitting parameters was selected to fit the concentration data. Meanwhile, a Formula (1) was obtained in an output report. C is the normalized concentration of a target compound at time t (month), and k is the first-order rate constant ($month^{-1}$). The time to dissipate 50% of a chemical (DT50) (half-life) was calculated in Formula (2) using the k value obtained in Formula (1). Linear regression was applied to determine the relationships between the biocide concentrations and soil properties including TOC and clay contents (%). Statistical analysis and dynamic curve fitting were performed using the software SPSS 13.0 and Sigma Plot 10.0, respectively.

$$C = C_o * \exp(-k * t) \quad (1)$$

$$DT50 = (\ln 2)/k. \quad (2)$$

3. Results

3.1. Azole biocides in the biosolid and biosolid-amended soils

The three target azole biocides climbazole, clotrimazole and miconazole were detected in a biosolid sample collected from Beijing WWTP at a concentration of 165 ± 6, 492 ± 21 and 427 ± 25 ng/g, respectively (three replicate samples). No significant losses were found during the storage.

The three azole biocides (climbazole, clotrimazole and miconazole) were detected in all the biosolid-amended soil samples (T1 and T2) collected from three sites in October 2010 (Tables S4 and S5), while they were not found in the soil samples from the control plots without the amendment of the biosolid (CK). It should be noted that the more biosolids, the higher pH values of the Zhejiang and Hunan soils were found and close to 7 (Table 2). Since the CK soils of Shandong were neutral (Table 2), the pH values were nearly similar.

Climbazole was determined at a mean concentration ranging between 0.6 and 4.3 ng/g for T1 and between 3.0 and 17.3 ng/g for T2 in October 2010, while clotrimazole was found in the concentration range of 2.2–8.3 ng/g for T1 and 15.3–41.0 ng/g for T2 in October 2010 (Fig. 1). The highest concentrations were detected for miconazole, ranging from 4.6 to 12.5 ng/g for T1 and from 27.0 to 64.5 ng/g for T2 in October 2010 (Fig. 1). For the three azole biocides found in the three sites for two treatments (T1 and T2), their concentrations in October 2010 followed the following order: miconazole > clotrimazole > climbazole. For T1 with the chemicals being aged in the biosolid-amended soils, the concentrations of these three biocides in the three sites had the following order Shandong > Hunan > Zhejiang, which may be related to the climatic and soil conditions of the sites (Table S4). No similar order was observed for T2, possibly due to the repeated applications. The concentrations for the three biocides were found to be related to organic carbon contents

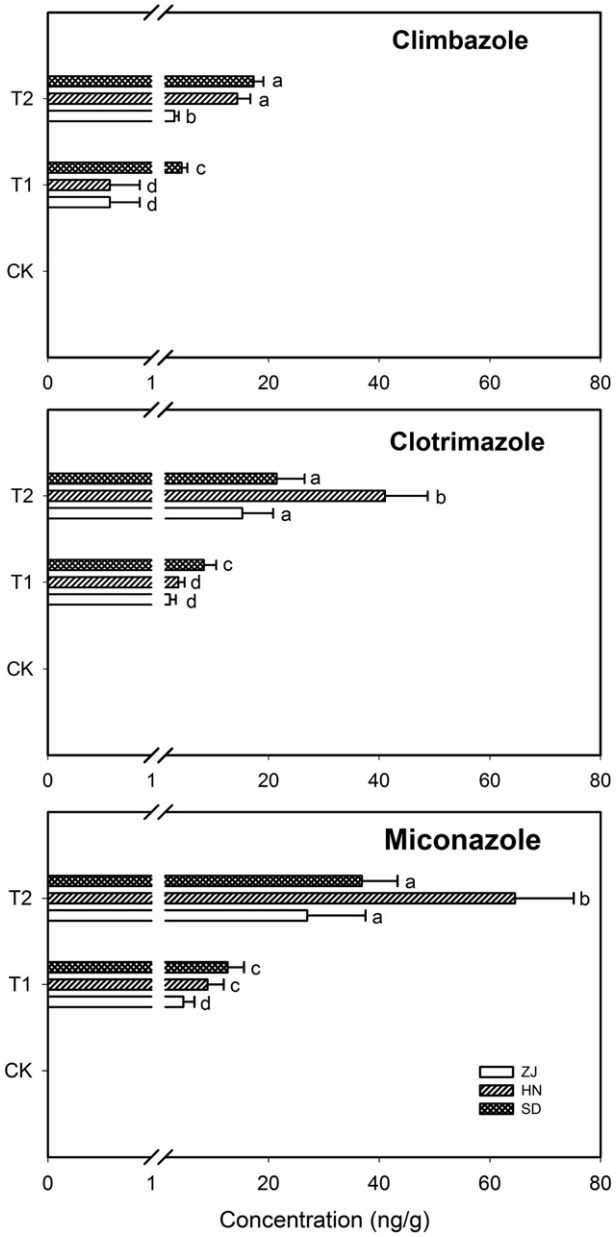


Fig. 1. Concentrations of the three biocides climbazole, clotrimazole and miconazole in the soils collected from the three trial sites Zhejiang (ZJ), Hunan (HN) and Shandong (SD) in October 2010. CK, T1 and T2 represent control, treatment 1 with one biosolid application, and treatment 2 with biosolid application every year. Letters (a, b, c and d) indicate the significant difference of concentration data by Duncan's multiple range test, $p < 0.05$. Error bars are standard deviations of the concentrations.

under both T1 and T2 in the soils, but not related to the clay contents (Fig. 2).

3.2. Field dissipation of the biocides in the biosolid-amended soils

The dissipation of the three biocides was assessed only for the Shandong trial site from October 2010 to October 2011 (Fig. 3; Tables S4 and S5). For T1 with only one biosolid application in May 2007, the three biocides had been aged in the Shandong site for more than three years before the initial monitoring on the 5th October 2010. The concentrations for the three biocides in the T1 soils varied from 4.3 to 3.8 ng/g for climbazole, 8.3 to 6.5 ng/g for clotrimazole and 12.5 to 7.4 ng/g for miconazole within the monitoring year. The fit of the concentration data to the first order model showed a very slow dissipation trend for clotrimazole and miconazole with their half-lives of 1019 ± 472 and 440 ± 75 days which represented the extrapolated values (Table 3; Fig. 3a and b). For climbazole, no significant dissipation was observed ($p > 0.05$). It should be noted that the fit for the concentrations of clotrimazole was not good ($R^2 = 0.1356$) so that the half-lives of clotrimazole was unreliable.

For T2, biosolids were applied again after the collection of the soil samples in October 2010. Big variations were observed during the period, which was attributed to the difficulty in obtaining homogeneous samples as shown in a previous study (Langdon et al., 2012). The concentrations of each biocide in the biosolid-amended soils were found to increase slowly from October 2010, and reached the maximum values in March 2011 (frost period) (Fig. 3, Table S5). There is no seasonal trend observed in this field trial. However, it is unknown that the concentrations of each biocide in the biosolid-amended soils demonstrated an increasing trend during October 2010 to December 2010 after the biosolids are applied. A decreasing trend in the concentrations was observed from March 2011 till October 2011 (Fig. 3); therefore, the nonlinear regression was performed for the data from March 2011 to October 2011. The average concentrations during this period decreased from 35.3 to 33.9 ng/g for climbazole, 44.4 to 33.0 ng/g for clotrimazole and 50.5 to 42.6 ng/g for miconazole (Table S5). Due to no significant dissipation for climbazole and miconazole ($p > 0.05$), the dissipation half-life calculated only for clotrimazole was 365 ± 110 days which represented the extrapolated values (Table 3; Fig. 3c).

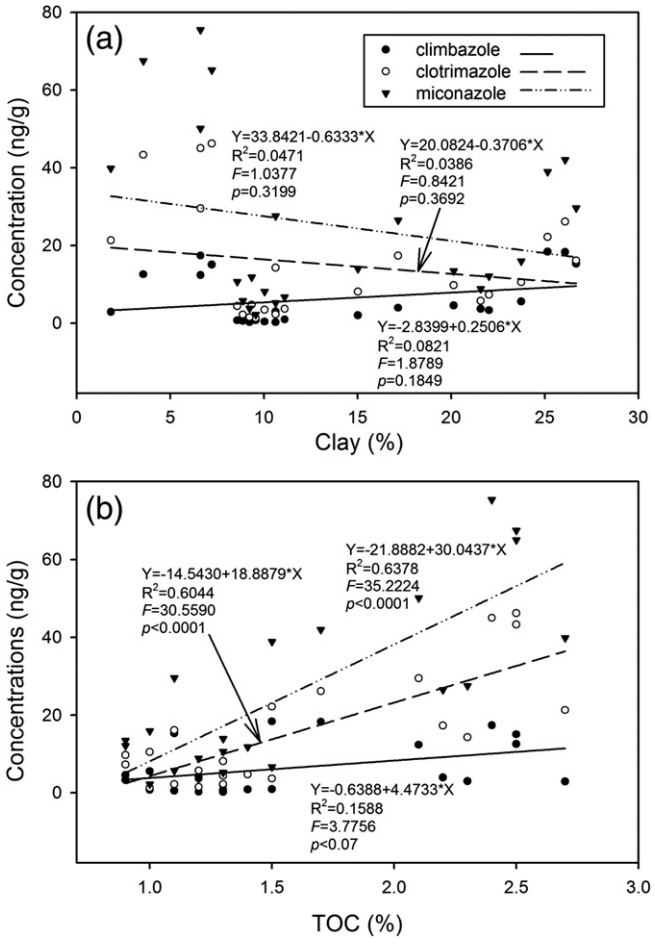


Fig. 2. Correlation analysis for the concentrations of the three biocides and (a) the clay content (%) and (b) total organic carbon content (TOC, %) of the biosolid-amended soils from the three sites in October 2010 (n = 24).

4. Discussion

The concentration levels of three biocides in the present study were in the same order of magnitude as found in digested dewatered sludge from other Chinese and Swedish WWTPs, with the maximum concentrations of 152, 426 and 970 ng/g for climbazole, clotrimazole and miconazole (Chen et al., 2012; Lindberg et al., 2010). Miconazole was found in biosolid-amended soils at concentrations of approximately 30–90 ng/g in United States and 150–340 ng/g in Canada (Gottschall et al., 2012; Walters et al., 2010). The previous studies reported that clotrimazole and miconazole were not strongly affected by sludge digestion and not readily bio-transformed in sewage sludge (Lindberg et al., 2010; Peng et al., 2012). This suggests that clotrimazole and miconazole persist in sewage sludge processes, and these chemicals may also persist in the biosolid-amended soils.

The results from the present study showed slightly different concentration patterns among the three field trial sites (Shandong, Hunan and Zhejiang), with the lowest residue concentrations for the three biocides found in the Zhejiang site for both T1 and T2 (Fig. 1). This could be attributed to the different field conditions including climatic and soil factors. The Zhejiang and Hunan sites were located in the south part of China, while the Shandong site was in the north part of China with colder and drier climate. Furthermore, the Zhejiang site was a flooding paddy field, which led to relatively higher dissipation of the biocides. Soil moisture can enhance the biological and abiotic degradation of these biocides (Garcia-Valcarcel and Tadeo, 2012) and biocide leaching. However the laboratory study (Garcia-Valcarcel and Tadeo, 2012) also showed strong adsorption and little desorption of azole biocides in the soils, which is consistent with their high log K_{ow} values (Table 1). The present study also found good correlations between the concentration and soil properties such as TOC (Fig. 2b). Laboratory incubation experiments showed a faster dissipation of clotrimazole in a clay loam than in a loam (Sabourin et al., 2011). This is consistent with the present result that the lower concentrations of clotrimazole found in the Shandong site in comparison with the Hunan site for T2. Thus the field conditions in the Zhejiang site were more conducive to the dissipation of the three azole biocides in the soil.

The three azole biocides climbazole, clotrimazole and miconazole for both T1 and T2 were found to be very persistent in the biosolid-amended soils, with their half-lives more than 300 days (Table 3). High persistence was also found for miconazole with its half-lives of 347 days (Gottschall et al., 2012) and 1386 days (Walters et al., 2010) in the sludge-amended field soils. This high persistent character of these biocides may be due to their good bacteriostasis and antisepsis effect to inhibit microbial activity, which resulted in the weak biodegradation (Al-Ahmad et al., 1999). However, to date no previous field dissipation data have been reported for climbazole and clotrimazole. Previous laboratory studies have reported the dissipation half-lives of 29–126 days for clotrimazole (Garcia-Valcarcel and Tadeo, 2012; Sabourin et al., 2011), which were much lower than 365 days in the present study. Thus laboratory experimental data may overestimate field dissipation rates and inaccurately predict the field dissipation patterns. This is consistent with the results for

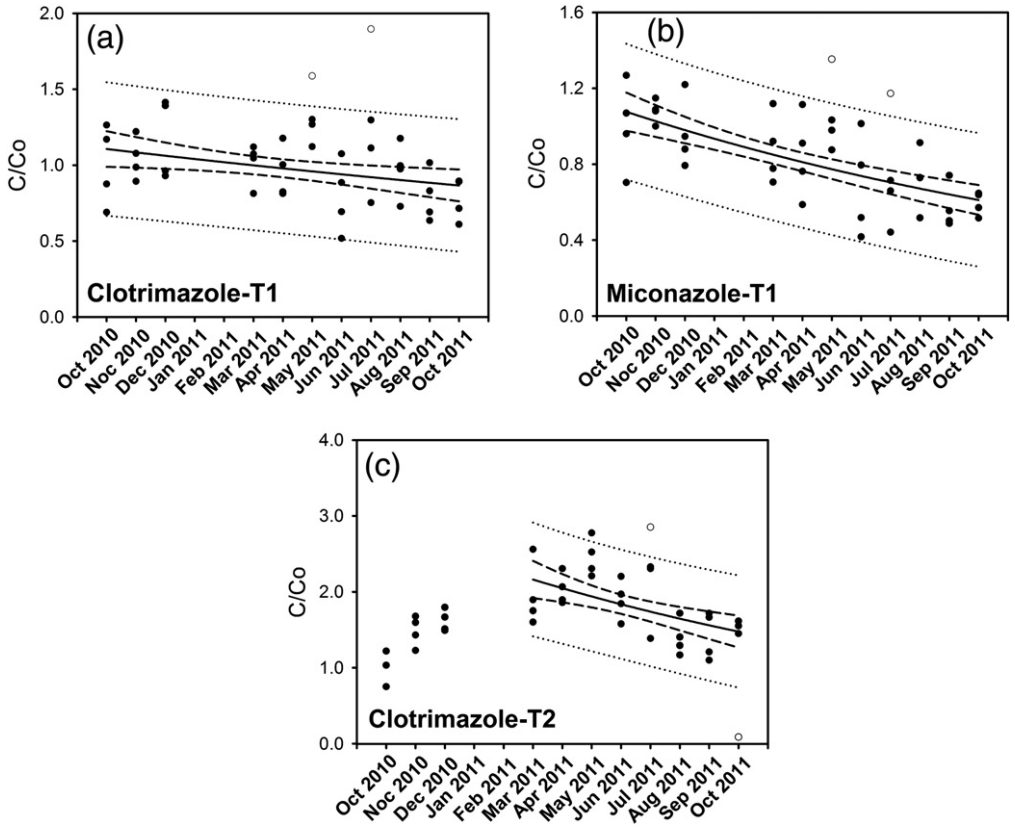


Fig. 3. Field dissipation of clotrimazole and miconazole in the biosolid-amended soils of the Shandong site within one year (October 2010 to October 2011). (a) Clotrimazole for T1; (b) miconazole for T1; and (c) clotrimazole for T2. All concentration data are normalized as a ratio of the concentration at each sampling time to the initial concentration (C/C_o). Data points with empty symbols are treated as outliers during data fitting since the points are not included between the two 95% prediction bands. The nonlinear regression fits for the first-order model, 95% confidence band and 95% prediction band are represented by the solid line, dash line and dotted line, respectively.

alkylphenols including nonylphenols and triclosan from Langdon et al. (2012) after comparing laboratory and field experiments.

With the Shandong site as an example, we predicted the concentrations of the three biocides in the soils based on the biosolid application rates and by assuming no dissipation following biosolid application, and compared the predicted concentrations with the measured concentrations in the soils collected in October 2010 (Table 4). For T1, the predicted concentrations could be regarded as the initial concentrations after biosolid application in May 2007. It is clear from the table that no marked differences existed between the predicted and measured concentrations, further suggesting very persistent nature of these three biocides in the biosolid-amended soils.

The presence of these azole biocides in the biosolid-amended soils suggests that biosolid is the pollution source for these biocides in the terrestrial environment. The persistence of these three biocides may lead to accumulation in soils and organisms in the environment. Uptake of these biocides by plants and bioaccumulation in earthworms from soils applied with biosolids is possible as shown for some pharmaceutical and personal care products (Kinney et al., 2008; Wu et al., 2010). A proper risk assessment for terrestrial organisms is not possible at this stage because of the lack of relevant toxicological data. Further studies are needed to explore potential risks to the environment and human health.

5. Conclusion

High persistence was found for the three biocides climbazole, clotrimazole and miconazole in the biosolid-amended soils of the three

Table 3
Summary of the dissipation information in biosolid-amended soils based on the first-order model for the three biocides in the Shandong site.

Compound	Calculation	T1	T2
Clotrimazole	Fitting formula	$Y = 1.1288 * \exp(-0.0204 * X)$	$Y = 3.1156 * \exp(-0.0570 * X)$
	R^2[a]	0.1356	0.3147
	p-Value[b]	0.0164	0.0013
	k (error)[c]	0.0204 (0.0080)	0.0570 (0.0159)
	DT50 (error)[d]	1019 (472)	365 (110)
Miconazole	Fitting formula	$Y = 1.1282 * \exp(-0.0472*X)$	–
	R^2	0.4717	
	p-Value	<0.0001	
	k (error)	0.0472 (0.0078)	
	DT50 (error)	440 (75)	

[a] The correlation coefficient of the first-order reaction kinetic model.
[b] Significance of the first-order reaction kinetic model.
[c] Rate constant of the first-order reaction kinetic model.
[d] The dissipation half-life (days) determined using the first-order reaction kinetic model under the two treatments (T1 and T2).

Table 4
Comparison of the predicted and measured concentrations (ng/g) for three detected biocides in biosolid-amended soils from the Shandong site.

Treatment	Biosolid application times	Climbazole		Clotrimazole		Miconazole	
		Predicted[a]	Measured[b]	Predicted	Measured	Predicted	Measured
T1	1	3.8	4.3	11.4	8.3	9.8	12.5
T2	3	11.4	17.3	34.0	21.5	29.6	36.8

[a] The predicted concentrations were calculated based on the measured concentration of corresponding biocides in the biosolid. Although the biosolid was stockpiled for four years and detected in 2010, the degradation of azole biocides in the biosolid was negligible due to their persistence (Lindberg et al., 2010; Peng et al., 2012). For both treatments (T1 and T2), the biosolid application rate is 60 t/ha per year. It is presumed that the plow depth is 20 cm and soil bulk density is 1.3 g/cm^3. We also presume that the biocides did not dissipate within 3 years.
[b] The measured concentrations were determined in the biosolid-amended soils collected in October 2010.

field trial sites including Zhejiang, Hunan and Shandong. No marked losses of the biocides were found three years following application with biosolid on the three sites, with the residual concentrations in a decreasing order: Shandong > Hunan > Zhejiang. The difference in residual concentrations among the three sites is related to the climatic and soil conditions. One year monitoring showed very slow dissipation of the three biocides in the Shandong site under both treatments with one biosolid application and successive yearly application, with their half-lives more than 300 days. Climbazole was more persistent than the other two biocides clotrimazole and miconazole in the biosolid-amended soils, with no significant dissipation for both two treatments. The environmental implications of the biocide residues in soils need to be investigated further in the future.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.03.004.

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