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INFLUENCE OF MOISTURE ON THE AVAILABILITY AND PERSISTENCE OF CLOTRIMAZOLE AND FLUCONAZOLE IN SLUDGE-AMENDED SOIL

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Abstract—Applying sewage sludge to soil is a common practice in many parts of the world. Thus, pharmaceutical compounds, such as azoles, can be released into the environment after sludge is applied to soil. To understand the fate of clotrimazole and fluconazole (pharmaceuticals used as antifungals in humans) in soil after its amendment with sludge, a reliable and sensitive method has been developed to determine these compounds in the solid and aqueous phases of soil. Desorption of clotrimazole from soil amended with sludge was negligible, whereas a rapid desorption of fluconazole was observed. Dissipation rates of these azoles were determined in amended soil incubated at 25°C with moisture contents ranging from 4.5 to 20%. Clotrimazole was more persistent than fluconazole in dry soil, whereas the contrary occurred in wet soil. Partitioning soil:soil solution of these azoles varied with time and moisture contents. Clotrimazole was found in soil with negligible amounts in soil solution, whereas fluconazole was approximately partitioned 50:50 during the assay time (60 d) at any soil moisture content. Occasional rainfall coupled with a relatively low binding soil capacity can result in the contamination of surface and groundwaters by fluconazole, whereas clotrimazole will remain in the soil. Environ. Toxicol. Chem. 2012;31:501–507. © 2011 SETAC

Keywords—Clotrimazole Fluconazole Persistence Availability Sludge

INTRODUCTION

Clotrimazole and fluconazole (Table 1) are antimycotic agents widely used in the treatment of fungal infections. These compounds block the sterol biosynthesis by inhibiting cytochrome P450-dependent 14α-demethylases. Clotrimazole is commonly administered in topical formulations, whereas fluconazole is administered topically and orally. After application, these azoles can be removed from the body by washing or through urinary excretion, which are the main entry pathways of azoles to municipal wastewater. Furthermore, the incomplete removal of azoles during wastewater treatment may be the origin of their presence in the effluent and sewage sludge of wastewater treatment plants. Thus, they may be introduced into the aquatic environment by waterways, or into the terrestrial environment by land application of sewage sludge, where they may cause toxic effects in nontarget organisms. Azoles are also inhibitors of the P450 aromatase, which catalyses the conversion of androgens to estrogens affecting sex differentiation in vertebrates exposed to these compounds [1,2]. Clotrimazole has been reported to show endocrine disruption activity in rainbow trout, frog, and salmon, as well as to affect photoprotective xanthophyll pigments in microalgal communities [2–5]. In addition, exposure to triazoles can cause skeletal defects and malformations in mouse embryos [6,7].

To monitor these azoles in the environment and understand their environmental behavior, reliable and sensitive analytical methods are needed. Determination of clotrimazole and fluconazole by gas chromatography–mass spectrometry (GC-MS) and liquid chromatography–tanden mass spectrometry (LC-MS/MS) in aquatic environmental samples have been reported in the last eight years [8–13], whereas only a few articles have been published on the analysis of these compounds in sludge by using LC-MS/MS, and where the presence of clotrimazole, and in some cases of fluconazole, in this matrix has been reported [12–14]. However, validated methods for the analysis of these azoles in soil or soil solution have not been found in the scientific literature.

The use of sludge as fertilizer in agriculture accounts for a high amount of the total municipal sludge disposal in many countries; thus, soil application of sludge is generally considered a sustainable practice. However, concerns exist with respect to the content and fate of organic contaminants present in the sludge, because its land application over the years could lead to an uncontrolled emission of organic pollutants into the environment that can remain for an extended period of time. In Europe, the pathogen and heavy metals content in sludge are regulated [15], but the levels of organic pollutants in sewage sludge have not yet been regulated in the European Union. Some European countries, however, have set limits for certain organic contaminants.

To evaluate the potential risk of an organic pollutant in sludge, it is not only necessary to know its levels in that matrix before soil application, but other factors have to be taken into account—such as soil properties, environment conditions, and sludge characteristics—which may affect the fate of pollutants in sludge-amended soil. The solid phase of soil represents an important component of the agronomic studies concerning the long-term assessment of the soil–plant system equilibrium, and of the evaluation of soil fertility. Another important component of the soil system is the soil solution, which is an efficient indicator of nutrient supply, with a chemical composition that directly reflects the balance between the soil solid phase and plants by constituting the interface where processes such as root absorption, soil chemical reactions, and solutes redistribution occur [16]. Moreover, terrestrial organisms are exposed to

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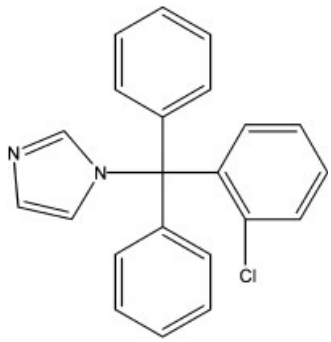
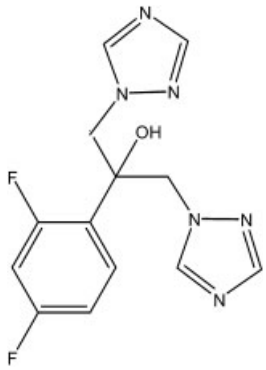
Table 1. Physico-chemical properties of studied azoles

	Clotrimazole	Fluconazole
Structure		
Vapor pressure (Pa)	3.31×10^{-7}	3.89×10^{-7}
Molecular weight (g/mol)	344.8	306.3
Water solubility (mg/L)	0.49	1.0
p*K*a	6.12	3.7
Log K_{OW}	4.1	0.25–0.4

chemical contaminants mainly through soil solution and, in addition, the soil solution can transport pollutants to surface water or groundwater.

The behavior of organic contaminants in soil depends on different dynamic physical, chemical, and biological processes that include sorption–desorption, volatilization, chemical and biological degradation, uptake by plants, run-off, and leaching. These processes govern the mobility of organic contaminants in soil, their bioavailability, and their transfer to other environmental compartments, such as the atmosphere and water [17], and are dependent on the physicochemical properties of pollutants, the environmental conditions, and the soil properties.

No data have been found in the literature regarding the behavior of the studied azoles in sludge-amended soil, and no analytical methods are available for their quantification in this matrix and in soil solution. The aim of the present work was to develop a reliable analytical method to quantify clotrimazole and fluconazole in these matrices, and to study the fate of these azoles in sludge-amended soil by determining desorption, dissipation rates, and changes with time of their levels in soil and soil solution under different soil moisture contents.

MATERIALS AND METHODS

Chemicals

Methanol of high-performance liquid chromatography grade was purchased from Scharlau Chemie. Silica-bondesil-C_{18}, 40 μm particle diameter, was obtained from Varian. Clotrimazole and fluconazole were purchased from Fluka & Riedel de Haën. High-performance liquid chromatography grade formic acid was purchased from Sigma, and deionized water was generated from a Q-pod water purifying system of Millipore.

Samples

Soil collected from the top layer (0–20 cm) of an agricultural sandy loam soil that had not been previously amended with sewage sludge was air-dried, sieved to pass 2-mm mesh, and analyzed according to official methods [18] to determine the main soil properties (sand:silt:clay ratios 64.8:23.9:11.3, organic matter content 0.98%, and pH 7.81).

Pellets of thermally dried sewage sludge were from a municipal wastewater treatment plant located in the city of Madrid, Spain. This material has an organic matter content of 50.16% and a pH of 7.74. Pellets were ground, fortified with clotrimazole, and fluconazole at 5 μg/g, using a standard mixture of 10 μg/ml, sieved (2 mm) twice, and finally allowed to equilibrate for 24 h at 4°C.

Soil was mixed with 3.6% fortified sewage sludge, which corresponds to a sludge dosage of 50 t/ha if the first 10 cm of soil are considered. This mixture was passed four times through a 2-mm mesh sieve to allow homogenization, and the nominal concentration of each azole in this mixture was 188 ng/g dry weight soil. The organic matter content of this mixture was 1.58% and the pH 7.74. Water field capacity, determined in a pressure chamber using a ceramic Plate [18], was 22% (w/w).

Desorption

Desorption of clotrimazole and fluconazole in soil amended with sludge (3.6% w/w) was determined after adsorption following the batch equilibrium of the Organisation for Economic Co-operation and Development Guideline Test 106 method [19], at room temperature. To previously adsorb these azoles, portions of 2 g of soil amended with nonfortified sludge was mixed, in triplicate, in a 50-ml Teflon centrifuge tube with 10 ml of 0.01 M $CaCl_2$ solution containing 5 μg/ml of clotrimazole and fluconazole. Centrifuge tubes were shaken on a mechanical shaker for 48 h to achieve soil equilibrium. Samples were then centrifuged at 6,000 rpm for 15 min to separate solid and aqueous phases, and 5 ml of supernatant was decanted and replaced by 5 ml of a 0.01 M $CaCl_2$ solution to desorb the azole compounds. This replacement was carried out once every 3 d, during a total period of 26 d. Supernatants were filtered and analyzed as indicated below. The remaining concentration in the solid phase after adsorption was calculated by the difference between the added amount and that determined in the aqueous phase.

Incubation experiments

Subsamples of soil amended with fortified sludge (6 g dry wt) were transferred to tared polypropylene columns (20 ml), with nonadsorbent polyethylene frits and paper filters at the

base. An adequate volume of water, determined by weighing, was added to each column to obtain a moisture content of 4.5, 11, and 20% (g/g dry wt), corresponding approximately to 20, 50, and 90% of the amended soil field capacity, respectively.

The columns containing the amended soil at the different moisture contents were placed in a glass desiccator with water at the bottom to avoid drying of the soil during the incubation period. The desiccator was placed in a chamber at $20 \pm 2^{\circ}C$ during the experimental period (60 d). Samples were taken after 0, 15, 30, 45, and 60 d with four replicates per soil moisture content. A blank of soil amended with nonfortified sludge was also sampled at each sampling time for preparation of matrix-matched standards, which were needed for the quantification of azole residues.

To obtain the soil solution, samples were mixed with water to raise soil moisture content to field capacity, and placed in the desiccator at 20°C for 48 h to allow equilibration. After this equilibration period, each column was weighed and its outlet inserted in a previously tared vial, which was placed and enclosed in a centrifugation tube. The whole unit was centrifuged at 4,000 rpm for 1 h at 18°C. After centrifugation, vials containing the soil solution and columns containing the amended soil were weighed separately.

Persistence

The concentrations of azoles in sludge-amended soil, which were incubated at different moisture contents, were plotted against time, and fitted to first order kinetics to determine the dissipation rate constants and half-lives for each azole using the equation

$$C = C_0 \cdot e^{-kt}$$

where C_0 is the initial concentration of clotrimazole or fluconazole (ng/g), C is the total concentration (concentration in soil plus concentration in soil solution) at time t (days) in ng/g, and k is the dissipation rate constant.

Half-lives of azoles were calculated using the k value, obtained by plotting the ln of the concentration against time, as follows

$$t_{1/2} = (\ln 2)/k$$

Analysis of azoles in soil and water samples

Water samples, soil solutions from the incubation assay, and supernatants from the desorption assay were analyzed directly by LC-MS/MS after filtration though a nylon filter of 0.45 μm mesh in the conditions described below.

Soil samples placed in polypropylene columns were extracted with methanol, based on a previously reported method for the determination of azoles in sludge [14]. Methanol (5 ml) was added to the column, which was placed in an ultrasonic bath where azoles were extracted during 15 min followed by solvent filtration in a multiport vacuum manifold. This procedure was repeated, and the extracts were combined and concentrated to 2 ml under a gentle stream of air at 30°C. To the concentrated extract, 0.1 g of C_{18} was added, the tubes were shaken for 1 min, and phases were allowed to separate for 10 min. An aliquot of the liquid phase was used for LC-MS/MS analysis.

Residues of clotrimazole and fluconazole in soil solutions and soil sample extracts were determined by an Agilent 6410 triple quadrupole tandem mass spectrometer interfaced by an electrospray ionization source, in positive mode, with an Agilent 1200 LC equipped with an autosampler, a quaternary pump, and a thermostated column.

Chromatographic separation of azoles in soil extracts and soil solutions were carried out using a Zorbax Eclipse XDB-C_{18} (150 mm × 4.6 mm i.d., 5-μm particle size) analytical column with a C_{18} security cartridge. The column was maintained at 40°C, and the injection volume was 10 μl. The mobile phase consisted of 0.04% acetic acid in water (solvent A) and methanol (solvent B). The elution gradient started at 90% solvent A with a flow rate of 0.3 ml/min, decreased to 50% A after 1 min to 0% A after an additional 5 min, at a flow rate of 0.6 ml/min. This was maintained for 4 min before solvent A was returned to the initial condition after 3 min, and the column was equilibrated for 5 min.

The ionization source parameters were as follows: drying gas flow 10 ml/min and 350°C, nebulizer pressure 40 psi, and capillary voltage 4.0 kV. Nitrogen was the gas used in the ionization source and in the collision energy cell.

Two MS-MS transitions for each compound were monitored during LC separation in the selected reaction monitoring, with a dwell time of 200 ms for each transition, one selected as quantifier and the other as qualifier. The ion transitions monitored for clotrimazole were 277.1 to 241.1 and 277.1 to 165.1, using a fragmentor voltage of 120 V and a collision energy of 30 V for both transitions; fluconazole transitions selected were 307.1 to 220.1 and 307.1 to 238.1, with a fragmentor voltage of 100 V and a collision energy of 20 V and 15 V, respectively.

To determine the matrix effect in the different sample extracts, blank extracts of soil and soil solution were obtained as described above at each sampling time and spiked with clotrimazole and fluconazole in the range of 0 to 400 ng/ml for soil solutions and from 0 to 700 ng/ml for soil samples. External standards were prepared in water or in methanol with a concentration of the target analytes in the same range, so that those employed in the matrix-matched calibration standards and their respective slopes from the calibration curves were compared.

The limits of detection (LOD) and limits of quantification (LOQ) of the method, calculated as the minimum amount of target analyte that produced a peak with a signal-to-noise response of 3 and 10, respectively, were determined for each analyte in the aqueous and solid matrices. Precision of the method was obtained by determining the relative standard deviations from repeated analysis of spiked extracts on the same day (repeatability) and on different days (reproducibility).

Recovery through the method was carried out with the soil amended with sludge used in the present study. Samples were fortified with clotrimazole and fluconazole before extraction at a level of 100 ng/g and analyzed as described above. Recoveries were calculated by comparing the MS/MS signal of sample extracts with that of a sample blank spiked at the same range of concentrations.

RESULTS AND DISCUSSION

Analytical determination

Good recoveries were obtained for both azoles in the analysis of soil samples amended with sludge (Table 2). The recovery of azoles from soil solutions or supernatants was not studied because they were analyzed by direct injection of aqueous samples. The detection and quantification limits were 5 ng/g (LOD) and 16.6 ng/g (LOQ), and 0.7 ng/ml (LOD) and 2.3 ng/ml (LOQ), for clotrimazole in sludge-amended soil and soil solution, respectively. Those for fluconazole were 0.5 ng/g (LOD)

Table 2. Analytical parameters of the developed method[a]

Soil	Recovery[b] (%)	LOD (ng/g)	LOQ (ng/g)	RSD (%) Intra-day	RSD (%) Inter-day
Clotrimazole	92.8 ± 3.1	5.0	16.6	4.3	5.0
Fluconazole	87.9 ± 3.2	0.5	1.7	3.9	6.1
Soil solution	**Recovery (%)**	**LOD (ng/ml)**	**LOQ (ng/ml)**	**RSD (%) Intra-day**	**RSD (%) Inter-day**
Clotrimazole	—	0.7	2.3	3.7	4.7
Fluconazole	—	0.3	1.0	1.6	3.3

[a] LOD = limit of detection; LOQ = limit of quantification; RSD = relative standard deviation.
[b] Four replicates.

and 1.7 ng/g (LOQ), and 0.3 ng/ml (LOD) and 1.0 ng/ml (LOQ), in sludge-amended soil and soil solution, respectively (Table 2). Reproducibility and repeatability were lower than 6% for both compounds (Table 2). Higher LOD and LOQ values for clotrimazole, in comparison with fluconazole, could be explained by the presence of this compound in the blank sample of sludge-amended soil, whereas no fluconazole was detected in that sample.

The presence of coextractants in sample extracts may produce a matrix effect that can suppress or, less frequently, enhance the signal of target analytes during their determination, affecting the accuracy of results. In the present study, the matrix effect was evaluated by comparing the slope of matrix-matched calibration curves in soil solution and in soil with the corresponding slope of calibration curves in water and methanol, respectively. Matrix effect was observed along with the assays in soil and soil solution samples, and this effect varied with the time of the assay as it is shown in Table 3. A higher matrix effect was observed in soil than in soil solution samples, and higher ion suppression was obtained in clotrimazole than in fluconazole, probably because of the higher lipophilic character of clotrimazole that originates at a higher retention time in the chromatographic analysis. This effect was somewhat higher at the beginning of the incubation assay, possibly because more organic compounds are extracted from soil recently amended with sludge than from aged amended soil. On the contrary, although an initial small matrix effect was observed in soil solutions, this effect showed an increase with time that could be explained by a higher extraction of soluble organic matter from amended soil along the assay. In view of these results, to compensate for the matrix effect observed in all cases, matrix-matched calibration curves were used at each incubation time for the quantification of azoles in sludge-amended soils and in soil solutions.

Table 3. Matrix effect in sludge-amended soil and in soil solution at different incubation times[a,b]

	Clotrimazole		Fluconazole	
	Soil % (SD)	Soil solution % (SD)	Soil % (SD)	Soil solution % (SD)
15 d	66 (3.3)	112 (4.1)	65 (3.9)	105 (3.5)
30 d	61 (3.0)	110 (5.2)	78 (4.7)	91 (3.0)
45 d	72 (2.9)	118 (4.4)	108 (4.2)	86 (1.4)
60 d	76 (3.1)	85 (3.1)	108 (6.5)	80 (1.3)

[a] Expressed as the percentage of the response obtained with matrix-matched standards compared with standards in neat solvent.
[b] Standard deviation (SD) is presented in parentheses for three replicates.

Desorption

As reversibility of adsorption plays an important role in the mobility of organic pollutants in the soil profile, desorption of clotrimazole and fluconazole was studied in the present work. In the multiple desorption steps, clotrimazole showed a very slow desorption, whereas desorption of fluconazole was fast (Fig. 1). After 3 d, 47% fluconazole previously adsorbed was desorbed, reaching a constant desorption rate after 12 d, with a cumulative release of approximately 90% of the initially adsorbed amount. This fluconazole behavior is similar to that reported for other triazole fungicides, such as hexaconazole, triadimefon, and penconazole, in soils where 30 to 60% of the triazole fungicide was retained after a single washing, depending on soil organic matter content that was the main factor observed in triazole adsorption [20]. On the contrary, clotrimazole desorption increased slowly with time and desorption continued after 26 d, without reaching an apparent desorption equilibrium.

The cumulative releases for fluconazole and clotrimazole after 26 d were approximately 94 and 4.4%, respectively. Therefore, clotrimazole is strongly adsorbed whereas fluconazole is weakly adsorbed in soil. Such differences were expected because of the high differences in K_{OW} values (Table 1), and can be attributed to the more polar character of fluconazole as

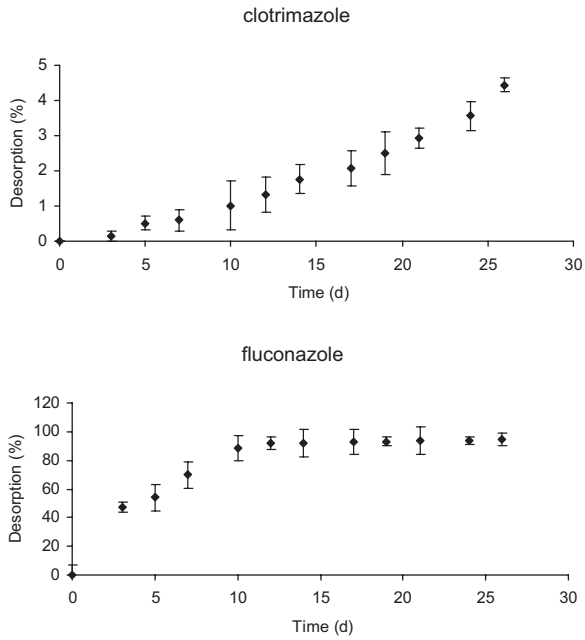


Fig. 1. Cumulative desorption of clotrimazole and fluconazole.

compared with clotrimazole, which has a higher affinity for organic surfaces, such as the sludge. The results in sludge-amended soils are in accordance with those of Kahle et al. [11], showing that clotrimazole is removed in wastewater treatment plants by sorption in sludge, whereas fluconazole loads in untreated wastewater were practically the same as in the corresponding effluent after wastewater treatment.

Persistence

A negligible dissipation by volatilization of target analytes was considered because of the low vapor pressure values of these compounds (Table 1). The obtained data showed a faster dissipation rate of clotrimazole at higher soil moisture contents, whereas for fluconazole no significant differences were observed (Table 4). These data are in agreement with the results reported for other organic compounds when soil moisture content was taken into account [21–23]. The influence of soil moisture content in the dissipation of organic compounds could be explained by their desorption from soil, which is smaller in dry soils than in wet soils, as pointed out previously [24]. A high and significant effect of soil moisture content in the dissipation rate was only observed for clotrimazole, with a considerably slower loss in dry soil than in moist soil, though an increase in soil moisture from 11 to 20% produced a negligible increase in the dissipation of this compound.

Half-lives for clotrimazole in soil varied between 126 and 29 d, whereas those for fluconazole changed between 85 and 73 d at low and high moisture content, respectively. According to the desorption values showed above, clotrimazole is more persistent than fluconazole at low soil moisture content (4.5%); nevertheless, the contrary occurs when soil moisture content is medium or high (Table 4).

Based on the bad fit to first-order kinetics of clotrimazole in dry soil, together with the high half-life value obtained (which was higher than the total time of the assay), this value should be considered as tentative. A similar half-life (114 ± 23 d) for a dry sandy loam soil was obtained by Sabourin et al. [25], although clotrimazole dissipation did not vary with moisture content. Nevertheless, it must be pointed out that the goodness of fit to the first-order kinetic model was poor (from 0.32–0.66).

It is commonly accepted that biodegradation occurs in soil solution because of the lower access of microorganisms in adsorbed compounds. However, the increase in organic carbon and microbial activity occurring from the application of sludge to soil may increase degradation because of a higher density of microorganisms, as well as increase in wet soil because of a higher availability of azoles in soil solution. On the other hand, differences in the dissipation rate of the studied azoles can be because of soil pH. Sorption properties of azoles can be modified by pH values that produce a dissociation of azole [26] and affect other soil properties, such as ion strength, influencing the degradation rate of azoles.

In the present study, the sludge-amended soil studied has an initial pH of 7.74 and varied with incubation time and moisture content, was approximately constant during the assay time (60 d) when amended soil was incubated at 4.5% of soil moisture content, and varied from 7.74 to 6.94 or from 7.74 to 7.02 when soil was incubated at 11 or 20%, respectively. The effect of soil moisture content in the dissipation of clotrimazole ($pKa = 6.2$) in comparison with fluconazole ($pKa = 3.2$) could be explained by the hydrolysis of clotrimazole, as it has been pointed out in a report from the Oslo and Paris Convention to Protect the Marine Environment of the North-East Atlantic Commission in 2005 [27], where an abiotic degradation of clotrimazole was suggested.

An additional determination of the dissipation rate was carried out with the control samples of sludge-amended soil, which contained an initial concentration of 31.3 ng/g clotrimazole (no fluconazole was detected in those samples), and were used as blanks for the matrix-matched calibration standards. The results obtained at the intermediate soil moisture content (11%) showed that the half-life of the native clotrimazole from the sludge (36 d) was similar to that of the freshly added clotrimazole (29 d). Nevertheless, the possible occurrence of nonextractable residues, particularly for clotrimazole [25], may affect the half-lives obtained.

Dynamics of azole levels in soil and soil solution

Changes with time of azole content in soil and soil solution, expressed as a percentage of initial content, are shown in Figure 2. The initial percentage of clotrimazole in soil at time 0, after equilibration at field capacity during 48 h, decreased with incubation time, and this decrease was faster at high moisture content. No significant differences on clotrimazole levels in soil were observed after 15 d of the assay at medium or high moisture content. At the end of the assay, after 60 d at constant soil moisture content, 68% of the initial amount was found in soil incubated at 4.5%, whereas approximately 26% was found in soil incubated at 11 or 20%. This represents variations from 188 to 124 ng/g at 4.5% soil moisture content and from 188 to about 50 ng/g at 11 or 20% soil moisture content.

Clotrimazole was present in soil solution at relatively insignificant concentrations, varying between 0.5 and 2% of the total amount added (Fig. 2). This variation represents levels between 5 and 19 ng/ml in soil solution. The low desorption of clotrimazole obtained in the desorption assay, together with the low levels observed in soil solution in the incubation assay and the relatively high dissipation rate when soil has a certain content of moisture, indicate its low availability to move to other soil compartments or leach into groundwater with the consequent low risk to aquatic organisms.

In the case of fluconazole, the initial content in soil at time 0, after equilibration at field capacity during 48 h, was 41.5% (64 ng/g) of the total amount added. An increase in the concentration in soil was then observed, varying between 55 and 63% of the total initial concentration, depending on soil moisture content during the first 15 d of the assay. These data show an initial redistribution of fluconazole into the solid matrix, probably because of migration and the diffusion of azole in soil as a function of the moisture content, making the fluconazole level in soil lower at low soil moisture content. After this time period, the adsorbed amount of fluconazole decreased approximately at the same rate for all moisture contents assayed. At the

Table 4. Dissipation constant (k) and half-life ($t_{½}$) values for fluconazole and clotrimazole in sludge-amended soil under different soil moisture contents

Azole	Moisture (%)	k	$t_{½}$ (d)[a]	R^2
Fluconazole	4.5	−0.0081	85 ± 3.5 B	0.9296
	11	−0.0085	81 ± 6.8 B	0.8061
	20	−0.0095	73 ± 2.3 B	0.9747
Clotrimazole	4.5	−0.0055	126 ± 20 A	0.8833
	11	−0.0236	29 ± 0.9 C	0.9827
	20	−0.0221	31 ± 2.7 C	0.9757

[a] Means with different letters are significantly different at 95% confidence level.

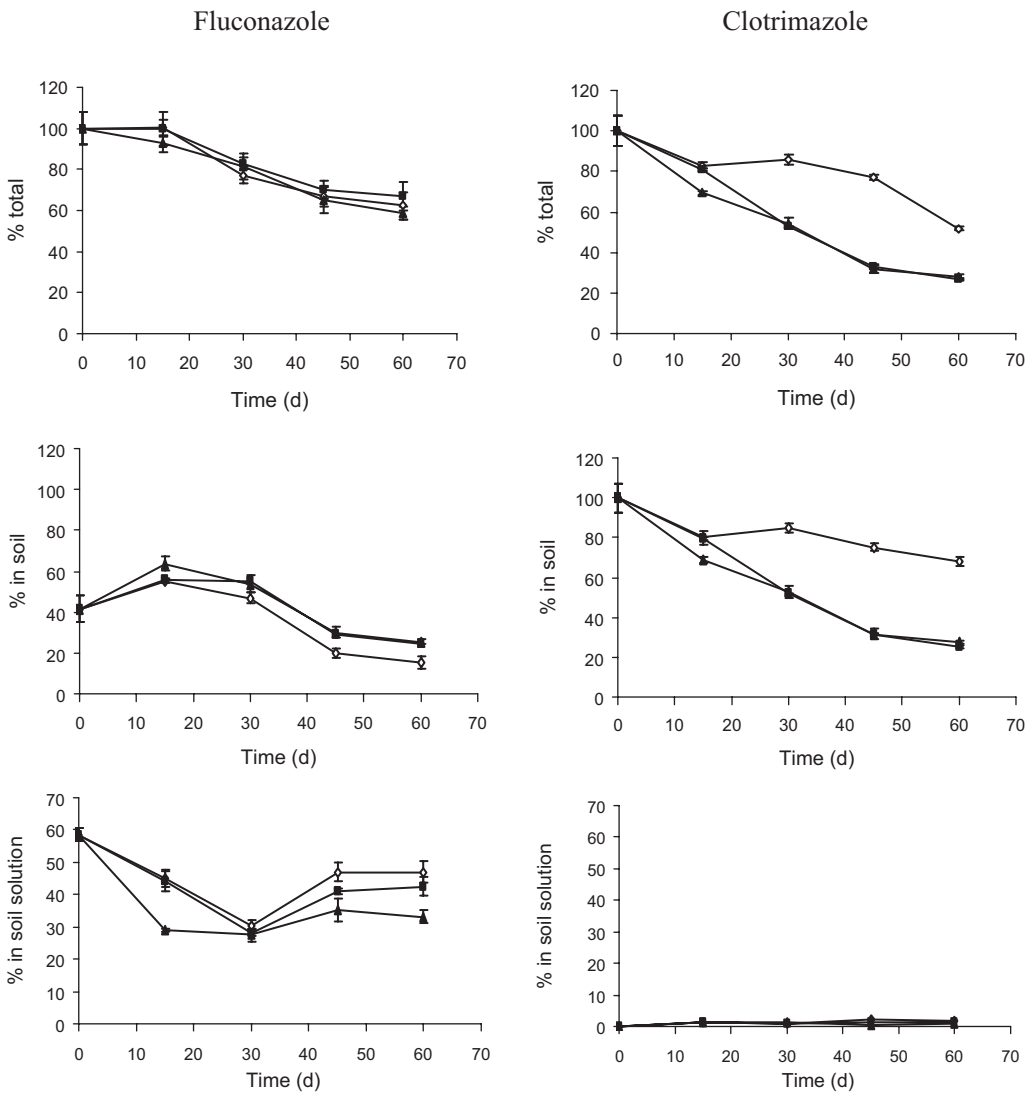


Fig. 2. Changes in total azole content, soil solution content, and soil content in sludge-amended soil incubated at different soil moisture contents: ◇ = 4.5%; ■ = 11%; ▲ = 20%. Values are the mean of four replicates (±SD) expressed as the percentage of initial azole content.

end of the assay (60 d), levels in soil were 15% (23 ng/g) and 25% (39 ng/g) of the initial amount added, for soil incubated at 4.5, and 11 or 20%, respectively.

The concentration of fluconazole in soil solution represented 58.5% of the initial amount added at time 0. This level decreased afterward for all incubation moistures, and similar fluconazole levels (about 27%) were found in soil solution for all the different moisture contents assayed at approximately 30 d of the assay. Then, from 30 to 45 d, a new increase of fluconazole in soil solution was observed, being high at low soil moisture content (Fig. 2) and remaining almost constant to the end of the assay. Levels in soil solution during the 2 months of the assay changed from 353 ng/ml to 259 ng/ml, and 238 ng/ml or 183 ng/ml for amended soil incubated at 4.5, 11, and 20% soil moisture content, respectively. It can be expected that fluconazole moves through the soil and reaches surface and groundwater, where it may pose a risk to nontarget organisms.

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