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Antifungal medicines in the terrestrial environment: Levels in biosolids from England and Wales

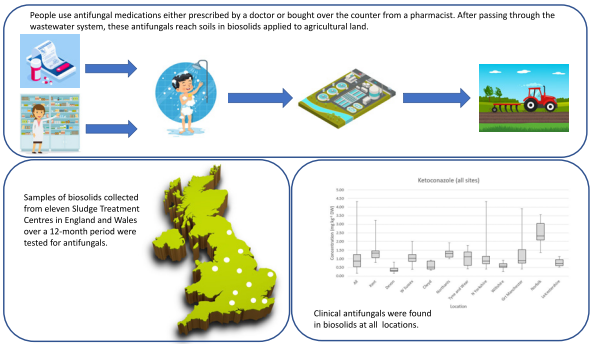
Ian Martin *, Alwyn Hart

Environment Agency, Horizon House, Deanery Road, Bristol BS1 5TL, United Kingdom of Great Britain and Northern Ireland

HIGHLIGHTS

- Clinical antifungals found in biosolids at treatment centres in England and Wales
- Over-the-counter items including ketoconazole and miconazole were most prevalent.
- Substances introduced at levels that may induce selection pressure for resistance.

GRAPHICAL ABSTRACT



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ABSTRACT

Antifungals are used widely in clinical and agricultural practice to control fungal growth, either treating or preventing infection. There are reports of increasing prevalence of resistance to antifungals in human pathogens and concern that their use in agriculture is driving clinical resistance in patients. While crop protection products are the most obvious source in agriculture, a further source may be biosolids from wastewater treatment. In the UK, these are applied to land to provide nutrients and improve soil structure for crops. In this study, biosolids from ten sites in England and one in Wales were analysed for clinical antifungals. Ketoconazole and miconazole were detected in all samples with a median concentration of 0.87 and 0.54 mg kg^{-1} dry weight (DW), respectively. Clotrimazole was detected at seven of eleven sites at a median level of 1.32 mg kg^{-1} DW and its absence at four others was considered treatment related. Two prescription-only and systemic medications, itraconazole and posaconazole, were frequently detected with median concentrations of 0.14 mg kg^{-1} DW and 0.09 mg kg^{-1} DW, respectively. The biosolid levels of itraconazole found in this study were two orders of magnitude higher than an indicative Predicted No Effect Concentration for resistance selection (PNEC-R) in soil. Neither fluconazole, griseofulvin, and voriconazole nor flucytosine and nystatin were found above the limit of detection of 0.01 or 0.1 mg kg^{-1} as received, respectively. The findings show that biosolids represent a viable pathway for antifungal agents to reach soil.

1. Introduction

Antimicrobial substances are used widely in clinical, veterinary, and agricultural practice to control the growth of microorganisms, either treating or preventing infection. Unfortunately, resistance to these substances is increasing in a wide range of pathogens. This antimicrobial resistance (AMR) may often result from the selection pressure created by antimicrobial usage as microorganisms evolve to become either more or fully resistant to the effects of antimicrobial agents that previously treated it effectively (Nelson et al., 2019). Antibiotic resistance in bacteria (Singer et al., 2016; Thanner et al., 2016), is often synonymous with AMR, however, other

* Corresponding author.
E-mail address: ian.martin@environment-agency.gov.uk (I. Martin).

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microorganisms can also develop resistance, and the rapid emergence of clinical antifungal resistance in pathogenic fungi poses a serious risk to disease control (Cohen and Denning, 2017; Fisher et al., 2018).

Members of the kingdom fungi occupy and provide a vast range of ecosystems and ecosystem services. Many species play a fundamental ecological role as saprophytes, releasing and recycling nutrients from dead organic matter (Fisher et al., 2020). Furthermore, yeasts and molds inhabiting the human digestive tract play a vital role in our microbiome (Arastehfar et al., 2020). Fungal metabolites are also an important source of medicines including antibiotics and immunosuppressants. However, some fungi are also the cause of human, animal, and plant diseases. In the case of fungal pathogens of humans, most infections have superficial outcomes, including the one billion people affected globally with skin, hair, and nail infections (Fisher et al., 2020). Nevertheless, global mortality rates for more serious conditions now exceed those of malaria or breast cancer and are comparable to those for tuberculosis and HIV (Brown et al., 2012). Approximately 90 % of deaths, around one and a half million globally each year, are due to fungal infections caused by *Aspergillus* spp., *Cryptococcus neoformans*, *Candida* spp., and *Pneumocytis jirovecii* (Arastehfar et al., 2020; Brauer et al., 2019; Fisher et al., 2020).

In terms of human antifungal therapies there are three main groups of substances that are used: azoles (collectively imidazoles and triazoles), polyenes, and echinocandins (Arastehfar et al., 2020). Polyenes such as amphotericin B and nystatin were introduced in the 1950s for treatment of systemic infections. Available from the early 2000s, echinocandins such as caspofungin and micafungin are the first-line treatment for invasive candidiasis (Bal, 2010). Azole-based agents are the dominant class of medicines (Brauer et al., 2019; Fisher et al., 2018). Clotrimazole and miconazole were amongst the first azoles introduced for topical treatment at the end of the 1960s (Parker et al., 2014) with systemic treatments including fluconazole and itraconazole following more than two decades later.

Fungi adapt rapidly in response to selection pressures and there is growing concern about the rise in antifungal resistance in important fungal pathogens (Arastehfar et al., 2020, Fisher et al., 2018; Fisher et al., 2020). Azole-based clinical antifungals share a common mode of action with agricultural crop protection antifungals (Arastehfar et al., 2020), disrupting cell wall development through sequestering and blocking the biosynthesis of ergosterol. Soil is a potential environment enabling selection for a common mechanism of resistance in pathogenic fungi for azole-based antifungals because it receives direct application of agricultural azoles and indirect emissions of clinical azoles via biosolids from urban wastewater treatment (Berger et al., 2017; Sewell et al., 2019).[1] Azole-based fungicides account for 38 % of all pesticide treatment on arable farm crops (Ridley et al., 2020). About 3.6 million tonnes of biosolids as received (AR) are applied to between 150,000 and 250,000 ha each year in the UK (Black, 2016). Azole resistant *Aspergillus fumigatus* have been frequently detected in soils collected at various rural and urban locations in England and Wales (Bromley et al., 2014; Sewell et al., 2019; Tsitsopoulou et al., 2018). Genomic analysis of samples from across the UK and Ireland reported occurrences where azole-resistant isolates of near identical genotypes were obtained from clinical and environmental sources (Rhodes et al., 2022).

The National Health Service (NHS) is the largest provider of health services to the public in England and Wales, and is organised into primary, secondary, and tertiary care. Primary care is the first point of contact for the public and includes general practitioners (GPs), health visitors, and other community-based services. Secondary and tertiary services, which includes hospitals and specialist care, is normally accessed only after a GP referral. Many antifungal medications are prescribed by primary and secondary care with the data recorded and reported separately (NHS Digital 2019, 2020; RX Info, 2021). Unlike antibiotics, many antifungal medications for topical use are also available without a prescription in England and Wales and sale volumes represent a significant data gap (Datapharm UK, 2019, Hudson, 2001, NICE, 2020). The UK market for such over the counter (OTC) medicines was £2.5 billion in 2017 (Connelly, 2017), which included £50 million for treatment of *Candida albicans* infections. £82 million was spent on skin fungal treatments in 2016 with the largest brand worth £39 million (Connelly, 2017; Connelly, 2018). Active ingredients include ketoconazole (used in antidandruff shampoos at up to 2 % *w*/w), miconazole (used in creams and sprays at up to 2 % *w*/w to treat skin and nail infections including tinea pedis), and clotrimazole (used to treat oral and genital candidiasis at up to 10 % *w*/w in creams and 500 mg pessaries). Fluconazole is the only antifungal medication available OTC for systemic use with oral capsules up to 150 mg (Hudson, 2001, Datapharm UK, 2019). All topical treatments are washed off after use and will enter the wastewater system largely unchanged.

The aim of this study was to investigate the levels of antifungal medicines in biosolids in England and Wales as a starting point to assess the significance of this pathway in delivering active antifungals to soil and potentially driving selection pressures in soil.

2. Materials and methods

The Chemical Investigations Programme (CIP) is an investigation and monitoring programme undertaken by the water and sewerage companies in England, Scotland, and Wales in collaboration with regulators and coordinated by the UK Water Industry Research Ltd. (Jones et al., 2014). An important element of the work from 2019 to 2022 has been to better understand the occurrence, behaviour, and management of trace and emerging contaminants in the sewer catchment. This included sampling of biosolids at 11 sludge treatment centres (STC) in England and Wales for a period of up to 12 months.[2] Samples were collected from biosolids ready for agricultural landspreading rather than from sludges at an intermediate point in sludge processing. In total, 202 samples were collected at sites in England and Wales between August 2020 and October 2021. In most cases, samples were collected monthly, but this was subject to operational constraints. All samples were stored prior to chemical analyses at −18 °C for up to 12 months, which occurred after all samples had been collected.

Although the original CIP analytical suite for contaminants was extensive, it did not include antifungals. This additional analytical work was carried out at the request of the Environment Agency as part of the Pathogen Surveillance in Agriculture, Food and the Environment (PATH-SAFE) programme (FSA, 2022) to investigate surveillance of environmental AMR and was undertaken on the biosolid samples after the initial CIP analysis had been completed. All analysis was undertaken by a commercial laboratory.

The clinical antifungals investigated by this project, the analytical approach, and the method limit of detection (LOD) on an as received (AR) basis is summarised in Table 1. The list of active ingredients was identified from a review of NHS prescription data (NHS Digital 2019, 2020; RX Info, 2021). Most medicines with the exception of amorolfine and clotrimazole are used systemically, but ketoconazole, miconazole, nystatin and terbinafine are also available OTC for topical use. In addition, the veterinary medicine enilconazole (CAS No. 35554-44-0) was also included because it is primarily used in the topical treatment of domestic animals including cats and dogs. Finally, climbazole was also included because of its use as a preservative in personal care products.

Analytical results were originally reported in biosolid samples on an AR basis, but they have been converted to dry weight (DW) in this paper using the reported dry solids content of individual biosolid samples (ranging from 21.3 to 68.0 %, median 30.3 %) reported by the laboratory.

3. Results

Clinical antifungals were detected in all samples analysed with those available in OTC medications, mainly for topical use, reported in most

[1] Biosolids is the term used to describe treated sewage sludge supplied to farmers for spreading onto agricultural land (ISO, 2020).

[2] About 53 million tonnes of untreated sludge is collected at around 8500 wastewater treatment works across the UK. The sludge is transported from smaller works to around 200 STC, often located at larger works.

Table 1
List of antifungal active ingredients included in analysis with the method limit of detection (LOD) reported on an as received (AR) basis and summary of extraction and analytical method.

	CAS no.	Use systemic (S) topical (T)	LOD mg kg^{-1} AR	Extraction method	Analytical method
Amorolfine	78613-35-1	Clinical: T	0.01	Acetonitrile was added to an aliquot of homogenised sample, fortified with Internal Standard, before shaking on a flatbed. The samples were subjected to a clean-up step using the Quick Easy Cheap Effective Rugged Safe method known as QuEChERS followed by a further dispersive solid phase extraction (dSPE). An aliquot of sample was transferred to a vial.	Ultra high-performance liquid chromatography coupled to triple quadrupole mass spectrometry using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6695 LC-QQQ in electrospray ionisation (ESI) mode. 5 mM Ammonium Acetate, 0.1 % Formic Acid in distilled water vs. 5 mM Ammonium Acetate, 0.1 % Formic Acid in Methanol. The separation column used was a reverse phase Agilent Eclipse Plus C18 with the dimensions of 50 mm × 2.1 mm, and spherical particle size of 1.8um.
Clotrimazole	23593-75-1	Clinical: T			
Enilconazole	35554-44-0	Veterinary: T			
Fluconazole	86386-73-4	Clinical: S			
Griseofulvin	126-07-8	Clinical: S, T			
Itraconazole	84625-61-6	Clinical: S			
Ketoconazole	65277-42-1	Clinical: S, T			
Miconazole	22916-47-8	Clinical: S, T			
Posaconazole	171228-49-2	Clinical: S			
Terbinafine	91,161–71-6	Clinical: S, T			
Voriconazole	137234-62-9	Clinical: S			
Climbazole	38083-17-9	Preservative	0.01	Acetonitrile and water were added to an aliquot of homogenised sample, fortified with Internal Standard, before shaking on a flatbed. The samples were then subjected to a QuEChERS clean-up, with dSPE (see above). An aliquot of sample was transferred to a vial.	
Flucytosine	2022-85-7	Clinical: S	0.1	Acetonitrile was added to an aliquot of homogenised sample, fortified with Internal Standard, before shaking on a flatbed. The samples were then subjected to a QuEChERS clean-up, with dSPE (see above). An aliquot of sample was transferred to a vial.	Ultra high-performance liquid chromatography coupled to triple quadrupole mass spectrometry using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6695 LC-QQQ in electrospray ionisation (ESI) mode. 0.1 % Formic in distilled water vs. 0.1 % Formic 90:10 Acetonitrile: distilled Water. The separation column used was a reversed phase Phenomenex Hydro RP with dimensions of 250 mm × 2 mm and a spherical particle size of 4um.
Nystatin	1400-61-9	Clinical: S, T	0.1	Formic acid in acetonitrile was added to an aliquot of homogenised sample, before shaking on a flatbed and centrifuging. An aliquot of sample was transferred to a vial.	

samples at individual sites. Conversely, many treatments available only with a prescription, primarily for systemic use, were not found above the method LOD in any samples. A summary of the main findings from the study are shown in Table 2.

Ketoconazole and miconazole were detected in all samples with a median concentration of 0.87 and 0.54 mg kg^{-1} DW, respectively. The observed levels of these substances were reasonably consistent across all sites (Fig. 1a and b). Clotrimazole had a frequency of detection for the whole data set of only 80 % and a median concentration of 0.94 mg kg^{-1} DW. However, there were differences observed between locations (see Fig. 1c). At seven sites, clotrimazole was detected in all samples with a median level of 1.32 mg kg^{-1} DW, while at the other four sites the detection frequency dropped to 40 % and a median concentration of 0.03 mg kg^{-1} DW. Terbinafine hydrochloride (HC) was also detected in 80 % of samples at a median concentration of 0.06 mg kg^{-1} DW. Although available OTC, neither amorolfine and fluconazole nor nystatin were found above the LOD of either 0.01 or 0.1 mg kg^{-1} AR, respectively.

Two prescription-only and systemic medications, itraconazole and posaconazole, were frequently detected. Itraconazole was found in 99 % of samples above the LOD with a median concentration of 0.14 mg kg^{-1} DW, while posaconazole was detected in 64 % of samples with a median concentration of 0.09 mg kg^{-1} DW. In both cases, the levels observed were broadly consistent across all locations (see Fig. 2a and b). Neither griseofulvin and voriconazole, nor flucytosine were detected in any samples above the method LOD of either 0.01 mg kg^{-1} AR or 0.1 mg kg^{-1} AR, respectively.

Table 2
Levels of antifungals determined in biosolids with samples from all sites combined (n = 201 unless stated). Detection frequency is based on a method LOD of 0.01 mg kg^{-1} AR (0.01–0.05 mg kg^{-1} DW).

	Detection frequency (%)	Median mg kg^{-1} DW	Mean mg kg^{-1} DW	Std. dev. mg kg^{-1} DW	Range mg kg^{-1} DW
Clotrimazole					
All sites (n = 201)	80	0.94	0.89	0.72	<0.03–2.59
7 sites (n = 133)[a]	100	1.32	1.32	0.47	0.39–2.59
Climbazole	76	0.06	0.07	0.05	<0.01–0.27
Enilconazole	17	0.04	0.04	0.02	<0.01–0.19
Itraconazole	99	0.14	0.15	0.08	<0.01–0.5
Ketoconazole	100	0.87	1.03	0.71	0.16–4.33
Miconazole	100	0.54	0.60	0.34	0.09–2.88
Posaconazole	64	0.09	0.15	0.19	<0.01–1.62
Terbinafine	80	0.06	0.06	0.03	<0.01–0.19

[a] Four of the eleven sites were known to have THP as a pretreatment for sludge prior to digestion and were associated with the lowest clotrimazole levels found across all sites (see text).

4. Discussion

Many antifungal medications are prescribed within the NHS by primary and secondary care with the data recorded and reported separately (NHS Digital 2019, 2020; RX Info, 2021). Table 3 presents an estimate of the amounts of clinical antifungals prescribed in England in a single month (July 2019), which amount to around one metric tonne. Primary care accounted for around 90 % of all prescribed antifungals. Unlike antibiotics, many antifungal medications are also available without a prescription in England and Wales and sale volumes represent a significant data gap (Datapharm UK, 2019, Hudson, 2001, NICE, 2020). Active ingredients in topical OTC medications were the most prevalent compounds detected in this study. This is broadly consistent with reports from elsewhere in Europe and beyond. Sludge concentration data for ten antifungals was summarised from the scientific literature by Sellier et al. (2022) with the median concentration for individual substances ranging 0.01–0.5 mg kg^{-1} DW. Clotrimazole, ketoconazole, and miconazole were the most prevalent. Median levels in activated sludge of 0.29 mg kg^{-1} DW, 1.60 mg kg^{-1} DW, and 0.17 mg kg^{-1} DW for clotrimazole, ketoconazole, and miconazole, respectively, were reported from Sweden (Östman et al., 2017). Ketoconazole was also found at 0.33 mg kg^{-1} DW in sludge samples from Germany (Chen and Ying, 2015). Itraconazole was found at levels of 0.05 and 0.15 mg kg^{-1} DW in biosolids from Galicia in north-west Spain (Castro et al., 2016). Miconazole was found at a median concentration of 0.3 mg kg^{-1} DW in biosolids (n = 24) from six Canadian wastewater

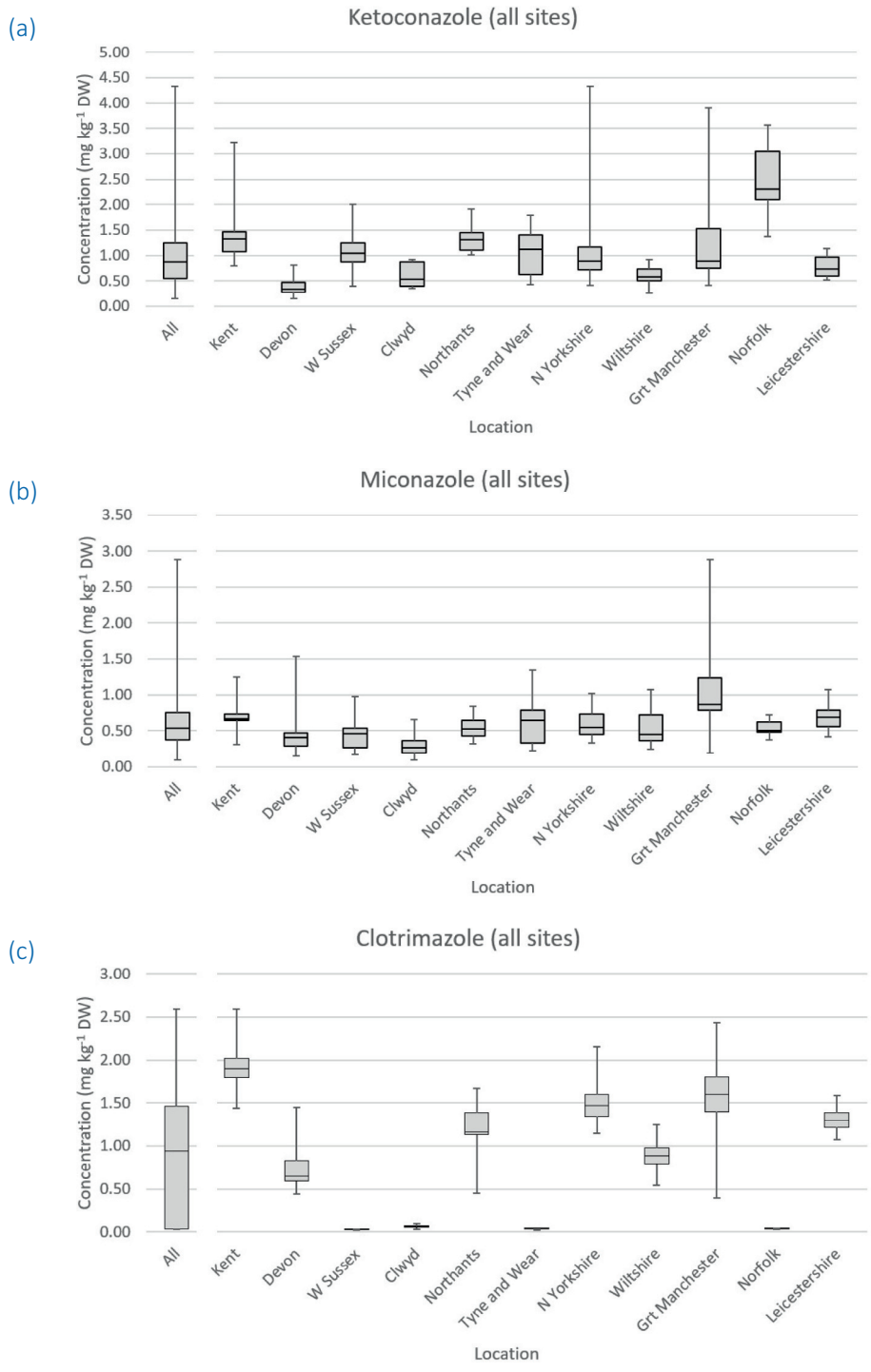

Fig. 1. Box plots of concentration data (LOD of 0.01 mg kg^{-1} AR) according to each sampling location (all times combined) for (a) ketoconazole, (b) miconazole, and (c) clotrimazole. These substances are available OTC for topical use.

treatment works (Guerra et al., 2014). Much lower levels of miconazole in the range 0.01–0.03 mg kg^{-1} DW were reported from sludges in the Czech Republic (Kodešová et al., 2019).

Although amorolfine, fluconazole, nystatin, and terbinafine HC are also available in England and Wales without a prescription (Datapharm UK, 2019), they were not found in appreciable amounts in biosolids in this study. Over the period 2013 to 2016, terbinafine HC was the most prescribed antifungal for systemic use in England, followed by fluconazole and itraconazole (PHE, 2017), accounting for 40 % by mass of antifungals prescribed in July 2019 (see Table 3). Only itraconazole was consistently detected in biosolids at levels >0.1 mg kg^{-1} DW. These findings in biosolids are consistent with data from activated sludge in Sweden, where fluconazole was not present above the method limit of quantification (LOQ) of 0.012 mg kg^{-1} DW. The median concentration of terbinafine HC was also reported as 0.082 mg kg^{-1} DW and 0.018 mg kg^{-1} DW in Sweden and Slovakia, respectively (Ivanová et al., 2019; Östman et al., 2017).

Understanding the observed variations between different antifungals and sites is complicated by the complexity of the treatment process. The samples collected at each STC are made up of treated sludges that came from smaller wastewater treatment works within the operational catchment. Partitioning and removal may have occurred during initial sludge treatment at these smaller works, such as by dewatering, as well as during

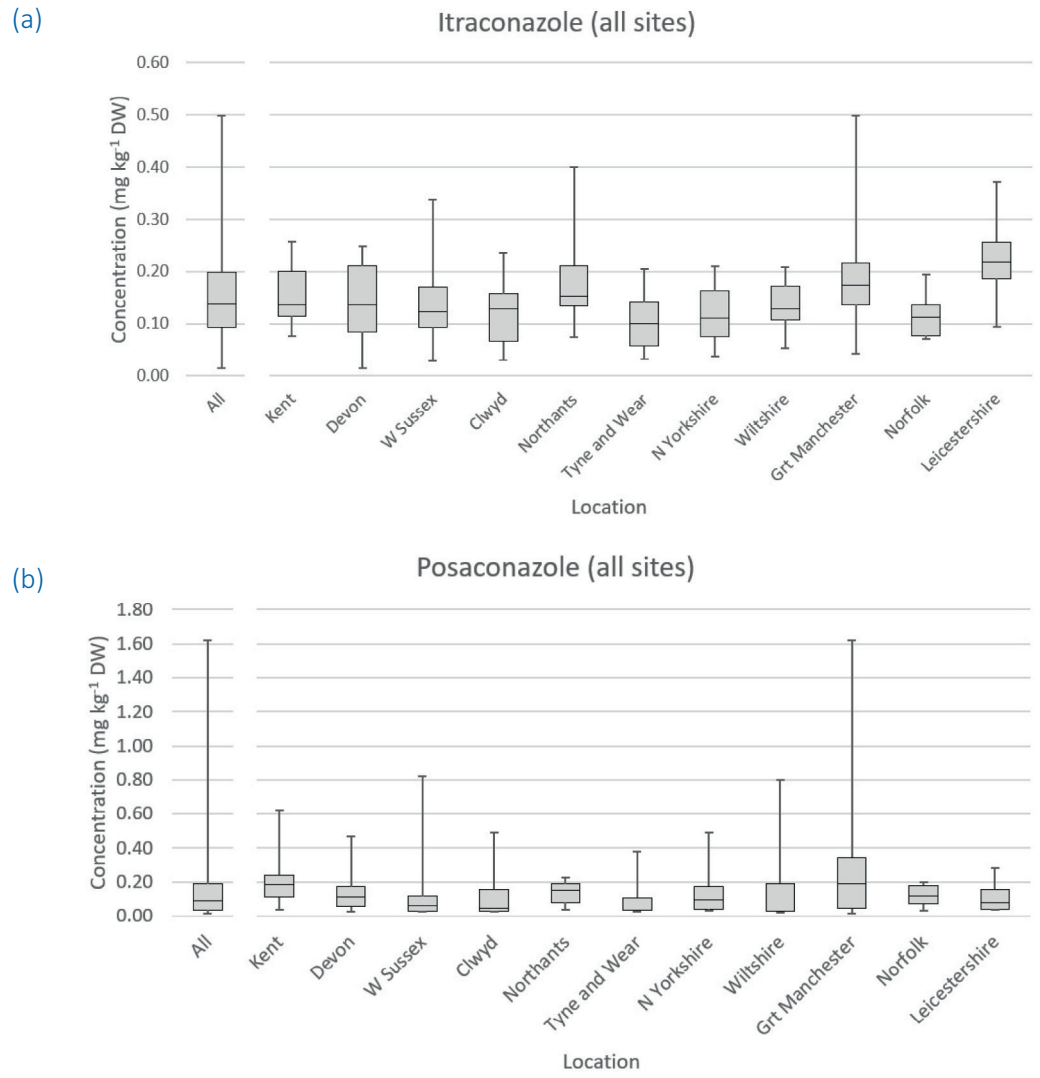

Fig. 2. Box plots of concentration data (LOD of 0.01 mg kg^{-1} AR) according to each sampling location (all times combined) for (a) itraconazole and (b) posaconazole. These substances are available only with a prescription and are for systemic use.

processing at the STC. Nonetheless, a possible explanation for some of the differences observed in the data is the degree to which these substances partition to sludge and their susceptibility to biotic and abiotic degradation at various points in the treatment process.

Table 3
Estimates of clinical antifungals prescribed by primary and secondary care services in NHS England in July 2019 (NHS Digital 2019, 2020; RX Info, 2021).

	Primary care amount (kg)	Secondary care amount (kg)	Total amount (kg)	Proportion of total amount prescribed (%)	Range found by this study (mg kg^{-1} DW)
Clotrimazole	83	10	93	8.7	<0.03–2.59
Fluconazole	30	19	49	4.6	<0.01
Itraconazole	38	6	44	4.1	<0.01–0.5
Ketoconazole	168	3	171	15.9	0.16–4.33
Miconazole	132	3	135	12.6	0.09–2.88
Nystatin	115	28	143	13.3	<0.1
Posaconazole	<1	15	15	1.4	<0.01–1.62
Terbinafine HC	412	4	416	38.7	<0.01–0.19
Voriconazole	<1	8	8	0.7	<0.01
Total prescribed			1074		

There is limited empirical data on the effectiveness of removal processes for antifungals (Liu et al., 2017; Östman et al., 2018; Wang et al., 2018) and an initial evaluation must rely on model predictions (for example, using the STPWIN module in EPI Suite).[3] In the case of fluconazole, STPWIN predicts that only 2 % of the influent concentration will partition to sludge, indicating that effluent levels are likely to be much more environmentally important. Terbinafine HC is predicted to partition predominantly to sludge, so the low levels reported in this study must involve other removal processes including degradation. One interesting feature of the biosolids data concerned clotrimazole, where it was the most prevalent antifungal medication at seven STC and all but absent at four others. Thermal hydrolysis processing (THP) as a pretreatment for sludge prior to anaerobic digestion is common at all four STCs with low clotrimazole levels in biosolids. This exposes sewage sludge to high temperature and pressure, and it seems probable that this treatment explains its absence at these sites rather than differences in catchment characteristics.

[3] The EPI (Estimation Programs Interface) Suite consists of several computer programs to estimate the physico-chemical properties and environmental fate of chemicals, which is made available by the United States Environmental Protection Agency (US EPA, 2020). STPWIN is a version of the fugacity-based Sewage Treatment Plant (STP) Model to predict the fate and transport of a chemical in a conventional wastewater treatment plant (Seth et al., 2008).

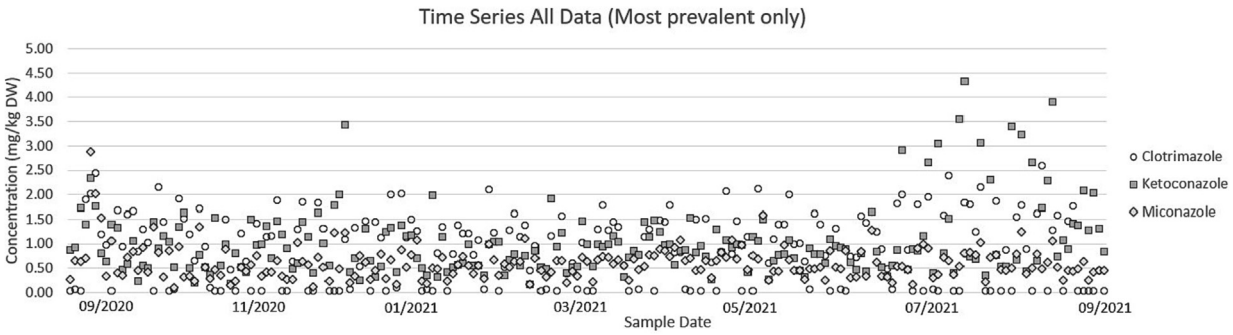

Fig. 3. Plot of the concentration data (LOD of 0.01 mg kg^{-1} AR) for clotrimazole, ketoconazole, and miconazole against sample time for the duration of the study (all site locations combined). There is visual evidence of an increased concentration of clotrimazole and ketoconazole in July and August 2021. These substances are available OTC for topical use.

More antifungal medications are traditionally used during the summer months compared to the winter, which contrasts with the seasonal peak observed with antibiotics (Curtis et al., 2019). This may be driven by treatment of skin and nail infections for aesthetic reasons when patients are likely to wear more revealing clothes and footwear (Adriaenssens et al., 2010). Fig. 3 shows the biosolid concentrations of the most prevalent antifungal medications found in this study plotted against the sample date from August 2020 to September 2021. Only for ketoconazole was a pronounced spike in concentrations observed in July and August 2021. Since the samples were in the final stages of treatment at the STCs, typically with a residence time of 4–6 weeks from initial sewage collection to biosolids, then this suggests elevated levels in influent occurred in May and June at the onset of summer in England and Wales. Notably, this effect decreased quickly in the early autumn.

Assessing the findings of this study represents a significant challenge. Direct quantification of enrichment is difficult, although a screening method has been proposed based on the minimal inhibitory concentration (MIC) of antimicrobials used in susceptibility testing. Bengtsson-Palme and Larsson (2016) proposed a predicted no effect concentration for resistance selection (PNEC-R) in water for a range of antimicrobials using the MIC data from the EUCAST database. Although this list was mainly antibiotics, they did propose a PNEC-R for several clinical antifungals, which included itraconazole (0.008 μg l^{-1}) and fluconazole (0.25 μg l^{-1}) from this study. However, fluconazole was not reported above the LOD in any of the biosolids sampled and is not considered further. Assuming the active chemical fraction for selection resistance in soil is in porewater, an indicative PNEC-R for itraconazole of 0.001 mg kg^{-1} DW can be derived for a generic soil using an equilibrium partitioning approach (ECHA, 2016). The median concentration of itraconazole in biosolids from this study was 0.14 mg kg^{-1} DW, which is more than two orders of magnitude higher than the indicative PNEC-R. Long-term repeat applications of biosolids at the concentrations observed in this study could also result in accumulated soil levels exceeding this screening threshold (depending on long-term fate and behaviour). In addition, levels of the most prevalent OTC substances in biosolids-amended soils could be in the same order of magnitude as long-term soil residues of agricultural fungicides. Silva et al. (2019) reported a median concentration of 0.02 mg kg^{-1} DW (n = 317, 24 % above method LOD) for the agricultural fungicide epoxiconazole, which was one of the most frequently detected pesticides in a study of European top soils.[4]

5. Conclusions

Antifungal medications were found to be routinely detected at levels close to 1 mg kg^{-1} DW in biosolids collected from eleven STC in England and Wales over a 12-month period. The most prevalent were azole-based and are found in products sold without a prescription for the treatment of various skin and nail infections, dandruff, and oral and genital candidiasis. Many of these topical treatments are likely to be washed off the skin after use and will enter the wastewater system largely unchanged. However, any relationship between usage and the observed levels is complicated by a lack of available data on the amounts sold OTC and the complexity of partitioning behaviour and abiotic/biotic removals within different treatment processes at individual STC.

Azole-based medicines have a common mode of action against fungi and are therefore susceptible to the development of resistance. Application of biosolids to agricultural land at the levels reported by this study, alongside use of fungicides for crop protection, may exert an environmental selective pressure that contributes to the emergence and promotion of antifungal resistance in biosolids and receiving soil. Further work is needed to characterise the hazard and the risk from the introduction of a wider range of clinical antifungals to soil, especially the more prevalent substances found in OTC, through (a) understanding the selection thresholds of concern including the development of PNEC-R based on MIC data and on experimental minimal selective concentrations (MSC) and (b) the fate of antifungals in soil including their persistence and mobility.

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CRediT authorship contribution statement

Conceptualisation, Ian Martin; Writing – Original draft preparation, Ian Martin; Writing – Review and editing, Alwyn Hart; All authors have read and agreed to the published version of the manuscript.

Data availability

Data will be made available on request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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[4] Expoxiconazole is a persistent azole-based fungicide used in the treatment of cereals.

The views expressed in this article are those of the authors and do not necessarily reflect the view or policies of the Environment Agency and no official endorsement should be inferred.

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