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Phytotoxicity of wastewater-born micropollutants – Characterisation of three antimycotics and a cationic surfactant

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ABSTRACT

Sewage sludge applied to soil may be a valuable fertiliser but can also introduce poorly degradable and highly adsorptive wastewater-born residues of pharmaceuticals and personal care products (PPCPs) to the soil, posing a potential risk to the receiving environment. Three azole antimycotics (climbazole, ketoconazole and fluconazole), and one quaternary ammonium compound (benzyldimethyldodecylammonium chloride, BDDA) that are frequently detected in municipal sewage sludge and/or treated wastewater were therefore characterised in their toxicity toward terrestrial (*Brassica napus*) and aquatic (*Lemna minor*) plants. Fluconazole and climbazole showed the greatest toxicity to *B. napus*, while toxicity of ketoconazole and BDDA was by one to two orders of magnitude lower. Sludge amendment to soil at an agriculturally realistic rate of 5 t/ha significantly reduced the bioconcentration of BDDA in *B. napus* shoots compared to tests without sludge amendment, although not significantly reducing phytotoxicity. Ketoconazole, fluconazole and BDDA proved to be very toxic to *L. minor* with median effective concentrations ranging from 55.7 μg/L to 969 μg/L. In aquatic as well as terrestrial plants, the investigated azoles exhibited growth-retarding symptoms presumably related to an interference with phytohormone synthesis as known for structurally similar fungicides used in agriculture. While all four substances exhibited considerable phytotoxicity, the effective concentrations were at least one order of magnitude higher than concentrations measured in sewage sludge and effluent. Based on preliminary hazard quotients, BDDA and climbazole appeared to be of greater environmental concern than the two pharmaceuticals fluconazole and ketoconazole.

1. Introduction

The safe and sustainable disposal of sewage sludge is an issue inherently related to wastewater treatment and the various options are discussed controversially. On the one hand, sewage sludge is continuously produced in wastewater treatment plants (WWTPs) and contains valuable nutrients and organic matter (Cogger et al., 2006); hence, a re-use in agriculture appears reasonable and is practised in many countries (Beecher, 2008). On the other hand, sewage sludge contains a variety of wastewater-born pollutants, especially those that are poorly degradable and sorptive, with often unknown environmental effects (Clarke and Smith, 2011).

Therefore, sludge application to land may transfer wastewater-born pollutants to soil and thus pose a risk to agricultural soil quality (Schowanek et al., 2004; Eriksson et al., 2008; Prosser et al., 2014). Less sorptive substances may also desorb from land-applied sludge, reach adjacent surface waters via particle-bound run-off (Topp et al., 2008) or be released to groundwater via irrigation with treated wastewater (Richter et al., 2015). Therefore, assessing the environmental risk of wastewater-born pollutants should take into account both terrestrial and aquatic organisms (Clarke and Smith, 2011; Langdon et al., 2010; Richter et al., 2015). Ecotoxicity data are particularly scarce for many sludge-associated micropollutants such as pharmaceuticals and ingredients of personal care products (PPCPs) (Topp et al., 2008; Clarke and Smith, 2011; Prosser et al., 2014; Chen and Ying, 2015).

One group of micropollutants frequently detected in wastewater are antimycotics containing an azole moiety (Garcia-Valcalrcel and Tadeo, 2012; Peng et al., 2012; Chen et al., 2013). Azoles are applied as fungicides in agriculture, but they are also used as active

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ingredients in cosmetics and pharmaceuticals. As demethylase inhibitors (DMI) azoles prevent the biosynthesis of ergosterol by inhibiting the cytochrome P450-dependent enzyme lanosterol 14-α-demethylase (CYP51) (Lepesheva and Waterman, 2007). However, many of them are known to also specifically interact with the hormonal balance of animals or plants (Rademacher, 2000).

Quaternary ammonia compounds (QACs) represent another micropollutant group detected at high concentrations in sludge (Ferk et al., 2007; Braguglia et al., 2014; Zhang et al., 2015). QACs are used in numerous consumer products such as detergents and PPCPs (Uhl et al., 2005) and in great quantities in the food and health care industries for cleaning and disinfection (Gerba, 2015). As cationic surfactants they disturb membranes but also interact with intracellular targets, exhibit genotoxicity or interact with the phytohormones of higher plants (Rademacher, 2000; Zhang et al., 2011; Gerba, 2015). Some QACs are therefore applied as plant growth regulators (Rademacher, 2000; Miliuviene et al., 2007).

From these two groups, four model substances were selected to represent various fields of application and different physicochemical properties with regard to their hydrophobicity and pH-dependent speciation (see Table 1). Ketoconazole, climbazole and fluconazole were selected to represent azoles that are contained in systemic antimycotics, anti-dandruff shampoos and skin creams, respectively. They have been detected in sewage sludge at concentrations of 50–4450 μg/kg dry weight (d.w.) (Garcia-Valcalrcel and Tadeo, 2011; Wick et al., 2010). As a QAC, BDDA (benzyldimethyldodecylammonium chloride) was selected which is detected at high concentrations in both effluent (up to 0.5 μg/L) and sewage sludge worldwide (up to 25 mg/kg d.w., Martínez-Carballo et al., 2007; Chen et al., 2014; Zhang et al., 2015).

Land application of sewage sludge can alter the chemical and physical properties of the soil, e.g. the organic matter content that can consequently affect the fate and effects of micropollutants in soil (Reid, 2000; Wu et al., 2009). Hence, testing their effects on soil organisms in the presence of a sludge matrix has been proposed as a more realistic approach compared to the commonly applied standard ecotoxicity tests with natural or artificial soil (Schowanek et al., 2004; Prosser et al., 2014).

The aim of the present study was therefore to i) determine the phytotoxicity of four wastewater-born PPCPs for which specific toxicity toward plants was assumed based on previous reports on structurally related substances (Rademacher, 2000; Miliuviene et al., 2007) using the aquatic plant *Lemna minor* and the terrestrial plant *Brassica napus*, and to ii) determine to which extent the presence of co-applied sludge would modify terrestrial phytotoxicity based on studies reporting an reduced pollutant availability (Wu et al., 2009; Holling et al., 2012; Peña et al., 2014; Prosser et al., 2014). Considering their log K_{ow} and pK_a (Table 1) the substances cover a range from hydrophilic (fluconazole), to medium and strongly hydrophobic properties (climbazole and ketoconazole, respectively). Based on the scheme given by Schaffer and Licha (2015), they should be uncharged (fluconazole), predominantly (climbazole and ketoconazole) or permanently cationic (BDDA) at the test medium or soil pH of about 7. As hydrophobicity drives partitioning to organic matter and cationic species strongly interact with negatively charged soil surfaces (Schaffer and Licha, 2015), the influence of sludge-amendment on phytotoxicity was expected to be greatest for ketoconazole and BDDA.

2. Materials and methods

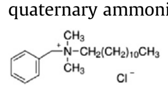
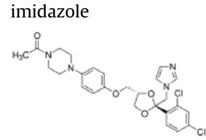
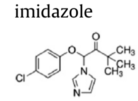
2.1. Test substances

BDDA (as "benzododecinium chloride") is registered as existing biocide (EC, 2014). In literature, also the synonym BAC-C12 is used (Clara et al., 2007). All BDDA concentrations in the present study refer to the cation (i.e. without the chloride moiety) as it is permanently dissociated in liquid and solid matrices and only the cation can be analytically determined. Fluconazole is used as antimycotic agent in the systemic and topical treatment of patients infected with various *Candida* species (EMA/888361/2011). Ketoconazole is a broad-spectrum antimycotic agent that has recently been suspended for oral but not for topical administration in the EU (EMA/CHMP/534845/2014). It is additionally registered as preservative in topical cosmetics and personal care products (with maximum concentration of 1% in rinse-off and leave-on products and of 2% in licenced anti-dandruff shampoos). Climbazole is likewise registered as preservative in topical cosmetics and personal care products (with maximum concentration of 0.5% in rinse-off and leave-on products and of 2% in anti-dandruff shampoos) and is registered under REACH with a production volume of 10–100 t/a (http://echa.europa.eu). Key characteristics of the test substances are compiled in Table 1.

2.2. Biotests with *L. minor*

The *L. minor* growth inhibition tests were conducted according to the OECD guideline 221 (OECD, 2006a) in modified Steinberg medium at 24 °C ± 2 °C under continuous photosynthetically active radiation (PAR) of 85–135 μE/(m^2s) (mean: 113 μE/(m^2s)) using

Table 1
Characteristics of the four test substances.

Substance	BDDA	Ketoconazole	Climbazole	Fluconazole
CAS No.	139-07-1	65277-42-1	38083-17-9	86386-73-4
Molecular weight [g/mol]	340 (cation: 304.5)	531	293	306
Log K_{ow}[a]	2.93	4.35	3.76	0.5
Log K_{oc}[a]	5.43	4.26	3.08	3.59
pK_a	not applicable	6.51/2.94[b]	7.5[c]	2.5; 2.9; 11.0[d]
Chemicalclass	quaternary ammonium compound	imidazole	imidazole	triazole
Structure				
Supplier of test item (purity)	Sigma–Aldrich Germany (99.0%)	TCI GmbH Germany (99.5%)	TCI GmbH Germany (99.9%)	TCI GmbH Germany (99.6%)

BDDA = benzyldimethyldodecylammonium chloride.
[a] EPISUITE, KOWWIN v1.68 and KOCWIN v2.0, MCI estimates.
[b] Skiba et al., 2000.
[c] Wick et al., 2010.
[d] Corrêa et al., 2012.