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Phytoremediation of imazalil and tebuconazole by four emergent wetland plant species in hydroponic medium

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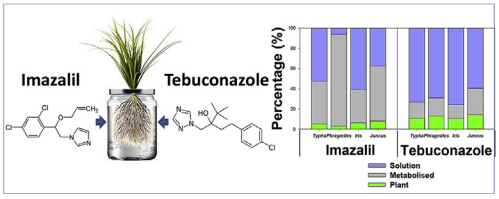
HIGHLIGHTS

- The imazalil and tebuconazole uptake abilities of four wetland plants are assessed.
- Removal of imazalil and tebuconazole follow a first-order removal kinetics model.
- Pesticide degradation is enantioselective in plants but not in solution.
- Pesticide phytoaccumulation depends on the compound hydrophobicity and plants.
- Phytoaccumulation of both pesticides occurred but is not a major removal mechanism.

GRAPHICAL ABSTRACT



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ABSTRACT

Pollution from pesticide residues in aquatic environments is of increasing concern. Imazalil and tebuconazole, two commonly used systemic pesticides, are water contaminants that can be removed by constructed wetlands. However, the phytoremediation capability of emergent wetland plants for imazalil and tebuconazole, especially the removal mechanisms involved, is poorly understood. This study compared the removal of both pesticides by four commonly used wetland plants, *Typha latifolia*, *Phragmites australis*, *Iris pseudacorus* and *Juncus effusus*, and aimed to understand the removal mechanisms involved. The plants were individually exposed to an initial concentration of 10 mg/L in hydroponic solution. At the end of the 24-day study period, the tebuconazole removal efficiencies were relatively lower (25%–41%) than those for imazalil (46%–96%) for all plant species studied. The removal of imazalil and tebuconazole fit a first-order kinetics model, with the exception of tebuconazole removal in solutions with *I. pseudacorus*. Changes in the enantiomeric fraction for imazalil and tebuconazole were detected in plant tissue but not in the hydroponic solutions; thus, the translocation and degradation processes were enantioselective in the plants. At the end of the study period, the accumulation of imazalil and tebuconazole in plant tissue was relatively low and constituted 2.8–14.4% of the total spiked

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pesticide in each vessel. Therefore, the studied plants were able to not only take up the pesticides but also metabolise them.

1. Introduction

Imazalil and tebuconazole are widely used as pesticides to control fungi in various crops, fruits and vegetables (SANCO, 2013; USEPA, 2002). Tebuconazole is also heavily used as a biocide for wood protection (Freeman et al., 2005), and imazalil can be used in the formulation of wood and building materials for protection products (Bylemans and Thys, 2007). Thus, imazalil and tebuconazole are commonly present in storm waters from urban settings (Bollmann et al., 2014). The structural and physicochemical properties of imazalil and tebuconazole are shown in Table S1 (supplementary material). The EU and USEPA have set maximum residue limits for both compounds in various food products (SANCO, 2013; USEPA, 2002). However, there is no formal environmental standard for either pesticide in water and aquatic ecosystems. Nevertheless, imazalil and tebuconazole pose a threat to aquatic environments. Castillo et al. (2006) found that low concentrations (1 μg/L) of imazalil damaged the composition of macroinvertebrate communities and that tebuconazole at 261 ng/L damaged non-target organisms (Campo et al., 2013).

Imazalil and tebuconazole are highly persistent (half-lives of 120–597 days) in soil (USEPA, 2002), moderately soluble in water (1400 and 36 mg/L for imazalil and tebuconazole, respectively) and can contaminate water resources through runoff. Phytoremediation, being one of the most environmentally sound and cost-effective methods for the decontamination and detoxification of pesticide-contaminated environments, is of great interest, especially in the form of constructed wetland systems (CWs) (Pilon-Smits, 2005). The uptake, accumulation, translocation and metabolism of micro-contaminants by plants have been suggested as important mechanisms for phytoremediation technology. Previous studies showed that wetland plants play an important role in the removal of nutrients (Brix et al., 2002), persistent organic pollutants (Carvalho et al., 2014), pharmaceuticals (Carvalho et al., 2014) and chemical industry organic pollutants (Lv et al., 2013). Thus far, only the aquatic macrophyte (*Elodea nuttallii*) is known to contribute significantly to the mitigation of imazalil (89 ± 3% removal) at a 93 μg/L influent concentration level (Stang et al., 2013), while *Typha latifolia, Leersia oryzoides, Sparganium americanum* (Moore et al., 2013) and *E. nuttallii* (Elsaesser et al., 2013) can significantly promote tebuconazole removal in CWs. However, these studies only report removal efficiencies, and to our knowledge, no studies have investigated the ability of emergent wetland plants to uptake and translocate imazalil and tebuconazole.

Many pesticides, including imazalil and tebuconazole, are chiral compounds. Chirality can be used to probe biological metabolic processes. Enantiomers usually differ in their biological properties, which may lead to differences in biodegradation rates and plant uptake (Chu et al., 2007; Wang et al., 2012). Monitoring the enantiomeric fraction is a very promising method to assess the significance of microbial degradation, given that other attenuation processes, such as dilution, diffusion, transport, and chemical reactions in the environment, are known to be non-enantioselective (Zipper et al., 1998). Thus, enantioselective analyses of imazalil and tebuconazole can help us better understand the mechanisms involved in pesticide removal processes.

In this study, we exposed four species of emergent wetland plants, namely, *T. latifolia (Typha)*, *Phragmites australis (Phragmites)*, *Iris pseudacorus (Iris)* and *Juncus effusus (Juncus)*, to 10 mg/L of the pesticides imazalil and tebuconazole in hydroponic solution for 24 days. Pesticide removal from the hydroponic solutions, as well as the removal kinetics, was assessed. Additionally, the imazalil and tebuconazole removal mechanisms, including the presence of enantioselective processes, plant uptake, translocation and accumulation, were also investigated.

2. Materials and methods

2.1. Plant material and experiment setup

The four species, *T. latifolia* (cattail), *P. australis* (common reed), *I. pseudacorus* (yellow flag) and *J. effusus* (soft rush), were propagated from seeds at the "Påskehøjgård" greenhouse facilities at Aarhus University, Denmark. After germination, individual seedlings were potted in 0.7-L pots containing a mixture (50:50) of sand and commercial peat-based potting compost. When the plants were approximately 200 mm tall, the soil was carefully washed off the roots, and the plants were rinsed in Milli-Q water. Four similarly sized plants from each species (6.2 ± 2.0 g fresh biomass) were selected and distributed randomly between the treatments. The plants were initially incubated for 10 days in hydroponic solution without pesticide to acclimate the plants to the hydroponic growth conditions. After the 10-day acclimation period, dead biomass was carefully removed, the plants were weighed and the hydroponic solutions were replaced. The treatments were (i) control (n = 3), (ii) 10 mg/L imazalil (n = 3) and (iii) 10 mg/L tebuconazole (n = 3). A 10-mg/L concentration level was used to ensure that the uptake levels could be measured and that the plant tissue analysis limitations could be overcome. The plants were mounted in the lids of 700-mL glass vessels using soft polyethylene foam, making sure that all roots were submerged in 500 mL of hydroponic solution, and then placed in a growth chamber (Bio 2000S, Weiss Umwelttechnik GmbH, Lindenstruth, Germany). The growth chamber was programmed at a 25:22 °C, 70:80% relative humidity and a 16:8 h light:dark cycle. The photon flux density at the base of the plants was approximately 400 μmol/m^2/s (PAR), as provided by metal halide bulbs. The vessels were covered with aluminium foil to ensure no light penetration into the solution.

In addition to the planted vessels, unplanted control vessels with 10 mg/L imazalil (n = 5) or 10 mg/L tebuconazole (n = 5) were placed in the growth chamber. The experiment ran for a period of 24 days, during which the hydroponic solutions were replenished daily to 500 mL with Milli-Q water (without imazalil and tebuconazole) to compensate for evapotranspiration and sampling losses.

The growth solution was prepared according to Smart and Barko (1985) and had the following composition (mg/L): Ca^{2+} 25.0; Mg^{2+} 6.8; Na^{+} 16.0; K^{+} 6.0; DIC 10.2; SO_4^{2-} 26.9; and Cl^{-} 44.2 (pH 7.7). Nitrogen, phosphorus and micronutrients were added daily using a commercial nutrient solution (Tropica Master Grow, Tropica Aquacare, Aarhus, Denmark) with the following concentrations (mg/L): N 1.64, P 0.12, K^{+} 1.26, Mg^{2+} 0.48, S^{2-} 1.11, B^{3+} 0.005, Cu^{2+} 0.007, Fe^{2+} 0.08, Mn^{2+} 0.05, Mo 0.002, Zn^{2+} 0.002.