ČESKÁ ZEMĚDĚLSKÁ UNIVERZITA V PRAZE
Fakulta agrobiologie, potravinových a přírodních zdrojů

KATEDRA
AGROENVIRONMENTÁLNÍ CHEMIE A VÝŽIVY ROSTLIN

SORPCE TEBUCONAZOLU NA KONTRASTNÍ TYPY PŮD A VYBRANÉ PŮDNÍ MINERÁLY

Doktorská disertační práce

Autor: Ing. Čadková Eva

Školitel: prof. Ing. Tlustoš Pavel, CSc.

Školitel specialista: doc. RNDr. Komárek Michael, Ph.D.

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Prohlášení

Prohlašuji, že jsem disertační práci na téma: „SORPCE TEBUCONAZOLU NA KONTRASTNÍ TYPY PŮD A VYBRANÉ PŮDNÍ MINERÁLY“ vypracovala samostatně a použila jen pramenů, které cituji a uvádím v přiloženém seznamu literatury.

V Praze dne 18. 6. 2012

Podpis

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Poděkování

Touto cestou bych chtěla poděkovat doc. Komárkovi a prof. Tlustošovi za vedení této disertační práce. Vřelé poděkování patří manželům Kaliszovým za cenné rady a připomínky. Děkuji také kolektivu katedry agroenvironmentální chemie a výživy rostlin a všem svým nejbližším za podporu, kterou mi prokazovali během studia a psaní této práce.

Obsah

1. Úvod

K největším problémům dnešní doby patří znečišťování životního prostředí. Mnohdy velice škodlivé látky vznikají jak přírodními procesy, tak antropogenní činností. Antropogenně vznikající látky jsou s ohledem na znečišťování životního prostředí považovány za problematičtější. Škodlivé látky migrují, popřípadě se akumulují v celém ekosystému zahrnujícím půdu, vodu, vzduch, ale i živé organismy. Příkladem lidské aktivity, která může znamenat riziko pro životní prostředí, je zemědělská činnost. Používání organických a anorganických prostředků k ochraně zemědělských plodin (pesticidů) je jednou ze zemědělských aktivit, při které může docházet k přesunu kontaminantů do životního prostředí. Aktivní substance mnoha těchto látek jsou těkavé, a tak končí v atmosféře. Používání pesticidů může dále vést ke kontaminaci půd. V závislosti na jejich fyzikálně-chemických vlastnostech a na vlastnostech daných půd může docházet k jejich akumulaci v půdách nebo jejich transportu v půdním profilu a popřípadě ke znečištění povrchových a podzemních vod. Je důležité podotknout, že v tomto případě je znečištění charakterizováno časovou prodlevou, než se látky dostanou do podzemních vod. U některých komponentů v pesticidech může být tato prodleva 20 až 30 let. Pesticidy se také mohou prostřednictvím rostlin dostat do potravinového řetězce a ohrozit tak kvalitu potravin a v konečném důsledku i lidské zdraví.

Mezi hojně používané látky k ochraně rostlin, osiv, ale i materiálů patří fungicidy ze skupiny triazolů. Jejich spotřeba každoročně vzrůstá. I přesto, že jsou tyto fungicidy aplikovány na rostliny, dostávají se do půdy právě při aplikacích nebo smyvem z rostlin. Často se jedná o látky, které se velmi dobře sorbují na půdní organickou hmotu, mohou se tak akumulovat v prostředí a negativně působit na půdní ekosystém. Nepředpokládá se, že by se tyto látky mohly snadno pohybovat půdním profilem a ohrožovat kvalitu podzemních a povrchových vod. I přesto byly naměřeny koncentrace fungicidů v povrchových vodách přesahující limity stanovené Evropskou unií pro pitnou vodu.

Dlouhodobě byly proti houbovým chorobám používány fungicidy na bázi mědi, což mělo za následek akumulaci mědi ve svrchních půdních horizontech. Jak bylo zjištěno, právě obsah mědi v půdě může výrazně ovlivňovat adsorpci organických fungicidů. V případě azolových fungicidů dochází k tvorbě Cu-azol komplexů, které ovlivňují sorpční schopnosti obou látek.

Proto je velmi důležité detailně prostudovat sorpční schopnosti a interakce těchto látek v půdním prostředí. Ze získaných údajů lze předpovídat jejich chování v půdním profilu a popř. tak omezit negativní dopady na životní prostředí.

2. Literární přehled

2.1 Vývoj fungicidních látek

Fungicidy jsou látky, které slouží k ničení nebo potlačování původců houbových chorob. V počátcích byly proti houbovým chorobám používány pouze anorganické fungicidy a téměř až do konce 19. století byla k potlačování padlí používána hlavně síra. Až v roce 1885 začal být ve Francii používán fungicidní přípravek Bordeauxská jícha, který byl hojně využíván proti padlí révovému a dalším chorobám. Tento přípravek byl kombinací síranu měďnatého a vápna. Přípravky na bázi organoměďnatých fungicidů, k ošetřování osiva proti sněti obilné, byly poprvé používány až v roce 1913 (Washington, 2002).

Od roku 1930 byly vyvíjeny synteticky připravené organické fungicidy. Mezi první synteticky připravené látky patří fungicidy ze skupiny dithiokarbamátů např. thiram, maneb, mancozeb (Washington, 2002).

K největšímu rozvoji organických fungicidů došlo v 60. a 70. letech 20. století, kdy byly představeny fungicidy ze skupin:

- benzimidazolů působících na široké spektrum vřeckatých hub (carbendazim)
- hydroxypyrimidinů působících proti padlí (dimethirimol)
- karboximidů působících proti rzi a sněti (oxycarboxin)
- dikarboximidů (ipridion)
- inhibitorů biosyntézy sterolů, které působí proti všem skupinám hub kromě oomycet (fenarimol, bitertanol, myclobutanil, propiconazole, triticonazole a další)
- fenylamidů působících proti saprolegnii a padlí
- anilinopyrimidinů působících proti plísni šedé a strupovitosti jablek

Většina zmíněných fungicidů patří mezi systémové fungicidy. Tyto látky jsou v rostlinách pouze částečně mobilní a pohybují se v rostlinách převážně xylémem. Výjimkou jsou fosforitany (fosetyl aluminium a kyselina fosforitá), které se v rostlinách mohou pohybovat vzestupně (xylémem) i sestupně (floémem) a působí převážně proti půdním patogenům.

Koncem 20. století začaly být vyvíjeny nové fungicidy na bázi přírodních látek např. strobiluriny (azoxystrobin, kresoxim methyl) (Washington, 2002).

2.2 Fungicidy patřící do skupiny azolů

Látky patřící do skupiny azolů (imidazoly, triazoly, diazoly) jsou hojně využívány v zemědělství na ochranu rostlin, ochranu materiálů např. dřeva, ale jsou také používány jako antimykotika v humánní i veterinární medicíně. Kvůli jejich rozlišnému používání v mnoha oblastech byly na tyto látky při jejich vývoji kladeny specifické požadavky. V humánní či veterinární medicíně byla požadována hlavně vysoká účinnost fungicidních látek a byl kladen důraz na to, aby účinné látky při léčbě pacienta nezpůsobovaly žádné vedlejší efekty. V zemědělství byla požadována vysoká specifická účinnost na patogenní organismy s důrazem na to, aby se tyto látky dále neakumulovaly v životním prostředí. Při ochraně materiálů byla vyžadována účinnost proti široké škále organismů, dlouhodobá stabilita látky a odolnost vůči alkalické hydrolýze (Bruns et al., 2005).

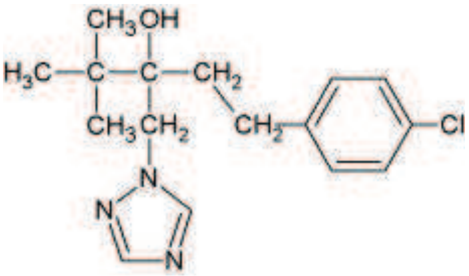
K největšímu rozvoji azolových fungicidů docházelo především v 70. a 80. letech 20. století. Množství používaných diazolových a triazolových fungicidů používaných k ochraně rostlin, ale i osiva, v posledních deseti letech významně vzrůstá jak v Evropě, tak na celém světě (FAOSTAT, 2011). Proto je velmi důležité pochopit jejich chování v životním prostředí a odhadnout a následně omezit možná rizika, která mohou být spojena s jejich aplikací. Tyto látky mohou být charakterizovány jako pětičlenné substituované aromatické heterocykly obsahující substituovaný a nesubstituovaný atom dusíku vzájemně v poloze 1,3 a případný třetí dusík je v poloze 1,2 k substituovanému dusíku. Na substituovaném dusíku je navázána lipofilní skupina zahrnující benzenový kruh. Fungicidní účinek azolů je primárně založen na inhibici biosyntézy ergosterolu, který je charakteristickým sterolem pro mnoho vyšších hub (ascomycetes, basidiomycetes, ale i funghi imperfecti). Inhibicí enzymu C-14 demethylázy, který katalyzuje 14-α-demethylaci lanosterolu, nedochází k přeměně lanosterolu v ergosterol. Následkem toho neobsahují plazmatické membrány hub ergosterol, čímž dochází ke změně pružnosti membrán a k omezenému růstu hub (Paulus, 2005). Hlavní roli při inhibici biosyntézy sterolů hraje nesubstituovaný atom dusíku v pozici 1,3, jenž komplexuje s atomem železa, který se nachází v protohemu, který je součástí cytochromu P-450 (koenzym demethylázy) (Gadher et al., 1983). Dále fungicidně působí i lipofilní skupiny, které jsou součástí azolových fungicidů. Ty jsou adsorbovány na povrchy buněk a mění tak permeabilitu membrán. Atomy chloru, které jsou vázány na benzenový kruh azolových fungicidů zlepšují rozpustnost účinných látek v lipidech (Paulus, 2005).

2.2.1 Tebuconazole

Tebuconazole [(*RS*)-1-*p*-chlorfenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl) pentan-3-ol] patří do skupiny triazolových fungicidů a je používán proti širokému spektru houbových patogenů (fómové hniloby brukvovitých, černi řepkové, hlízence obecné, fuzarióze klasů, padlí chmelovému, moniliové spále peckovin, moniliové hnilobě peckovin, rzi slivoně, sněti mazlavé, sněti prašné ječné, braničnatce plevové, padlí travnímu, rzi, hnědé skvrnitosti ječmene, rynchosporiové skvrnitosti, padlí révovému, plísni révové, plísni šedé, strupovitosti, atd.) a slouží k ochraně mnoha zemědělských plodin (obilnin, révy vinné, řepky olejky, chmele otáčivého, peckovin, slivoní, jádrovin, atd.) (Registr přípravků na ochranu rostlin, 2011). Jeho hlavní fyzikálně chemické vlastnosti jsou uvedeny v tabulce 1.

Účinná látka tebuconazole je obsažena v mnoha komerčně dostupných přípravcích (Cello, Falcon 460 EC, Hattrick, Horizon 250 EW, Lamardor FS 400, Lynx, Orius 25 EW, Orius 5 FS, Orius 6 FS, Ornament 250 EW, Prosaro 250 EC, Raxil 060 FS, Raxil 515 FS, Raxil ES, Raxil TNT, Scenic 080 FS, Staccato, Zamir 40 EW). Pro různé způsoby použití jsou na trhu přípravky s touto aktivní látkou k dispozici v mnoha formulačních úpravách (EW-emulze typu olej ve vodě, EC-emulgovatelný koncentrát, FS-kapalný suspenzní koncentrát pro moření osiva, DS-prášek k suchému moření osiva, SC-suspenzní koncentrát, WG-ve vodě dispergovatelné granule, WP-smáčitelný prášek) (FAO, 1994).

Tabulka 1. Fyzikálně-chemické vlastnosti tebuconazolu dle IUPAC (2011) a FAO (1994).

Chemický název	(*RS*)-1-*p*-chlorfenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol
CAS	107534-96-3
Chemická skupina	triazoly
Vzorec	
Molekulová relativní hmotnost	307,8
Rozpustnost ve vodě (g.l^{-1})	0,036 (při 20 °C)
Bod tání (°C)	105
log K$_{ow}$	3,7 (při 20 °C)
K$_{oc}$ (l.kg^{-1})	803–1251
Degradace v půdě (d)	55,8–365

2.3 Sorpce organických fungicidů v půdě

Organické fungicidy jsou používány při ochraně mnoha zemědělských plodin a právě jejich rozsáhlé používání může vést ke kontaminaci životního prostředí zahrnující kontaminaci půdy, ale i povrchových a podzemních vod. Hildebrandt et al. (2008) uvádějí, že koncentrace fungicidů v povrchových i podzemních vodách řek, které se nacházejí v zemědělsky aktivních oblastech, přesahovaly regulační limity (0,1 $\mu g.l^{-1}$) stanovené Evropskou unií pro pitnou vodu (EC, 1998).

Protože organické fungicidy patří do mnoha chemických skupin, je jejich adsorpce, transport a chování v životním prostředí ovlivněno hlavně jejich fyzikálně-chemickými vlastnostmi a vlastnostmi prostředí, ve kterém se nacházejí (Wheeler, 2002).

2.3.1 Fyzikálně-chemické vlastnosti fungicidů ovlivňující jejich sorpci v půdě

Mezi klíčové procesy, které ovlivňují koncentraci a pohyb pesticidů/fungicidů v půdním prostředí, patří adsorpce (Kamrin, 1997; Gevao et al., 2000; Wheeler, 2002; Huang et al., 2003; Arias-Estévez et al., 2008). Obecně platí, že sorpce fungicidů souvisí s jejich rozpustností ve vodě (S) ($g.l^{-1}$) (tabulka 2), s rozdělovacím koeficientem oktanol-voda (K_{ow}), distribučním koeficientem (K_d) a rozdělovacím koeficientem vztaženým na organický uhlík (K_{oc}) (Wheeler, 2002).

Rozdělovací koeficient K_{ow} je definován jako poměr rovnovážných koncentrací rozpuštěné látky (např. pesticidu) ve dvoufázovém systému dvou omezeně mísitelných rozpouštědel-oktanolem a vodou:

$$K_{ow} = \frac{C_o}{C_w}$$

kde C_o je koncentrace látky v n-oktanolu ($g.l^{-1}$) a C_w je koncentrace látky ve vodě ($g.l^{-1}$). Čím je hodnota K_{ow} vyšší, tím mají fungicidy větší schopnost sorbovat se v půdě a sedimentech či se kumulovat v biotě (hodnoty log K_{ow} vyšší než 7 či 8 indikují, že tyto látky jsou v prostředí téměř nemobilní a mají vysokou sorpční schopnost) (Chamberlain et al., 1996; Baird, 2003). Hodnota log K_{ow} také závisí na schopnosti pesticidu ionizovat se, což úzce souvisí s jeho konstantou kyselosti K_a (Chamberlain et al., 1996; Gevao et al., 2000).

Distribuční koeficient K_d ($l.g^{-1}$) popisuje distribuci fungicidu mezi pevnou a kapalnou fázi:

$$K_d = \frac{C_s}{C_e}$$

kde C_s je koncentrace sorbované látky v pevné fázi ($g.g^{-1}$), C_e je rovnovážná koncentrace látky v kapalné fázi ($g.l^{-1}$).

Vyšší hodnoty K_d indikují vyšší schopnost látky sorbovat se v půdě.

Obsah organické hmoty v půdě je jedním z hlavních faktorů, které mají vliv na sorpci azolových fungicidů. Proto je důležitým ukazatelem mobility fungicidů/pesticidů hodnota rozdělovacího koeficientu K_{oc} ($l.g^{-1}$), který je definován stejně jako koeficient K_d s tím, že distribuce fungicidu mezi pevnou a kapalnou část je vztažena na obsah organického uhlíku v dané půdě (Sukop a Cogger, 1992; Monkiedje a Spiteller, 2002; López-Blanco et al., 2005).

$$K_{oc} = \frac{K_d}{F_{oc}}$$

kde K_d je distribuční koeficient ($l.g^{-1}$), F_{oc} je frakce organického uhlíku v půdě (bezrozměrné).

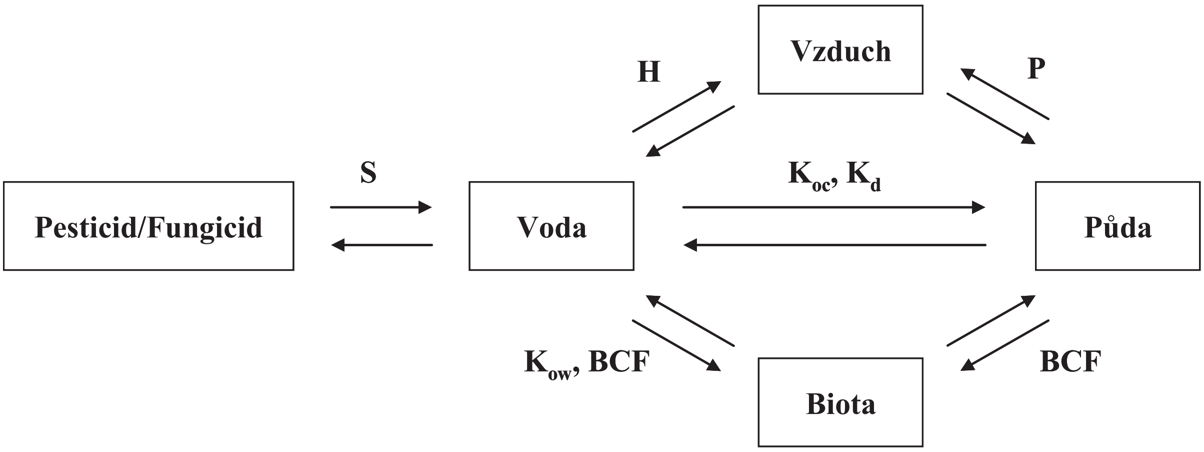
Mezi další faktory, jimiž lze popisovat pohyb fungicidů v celém ekosystému, patří tlak nasycených par (*P*), biokoncentrační faktor BCF a Henryho konstanta (*H*) (Wheeler, 2002); jejich vliv na chování fungicidů v životním prostředí je schematicky znázorněn na obrázku 1. Hodnota tlaku nasycených par vypovídá o schopnosti látky přecházet do plynné fáze. Obecně volatilita látek roste s teplotou a v půdě budou více zadržovány perzistentní látky s nízkým tlakem par. BCF je definován jako poměr koncentrace chemické látky nalezené v biotě ke koncentraci v zevním prostředí a je přímo úměrný hodnotám log K_{ow}. Henryho konstanta popisuje volatilitu látky z vodného roztoku. Čím je hodnota H vyšší, tím je látka těkavější a má vyšší tendenci přejít z vodního prostředí do atmosféry.

Tabulka 2. Rozdělení fungicidů dle rozpustnosti, mobility a jejich persistence v prostředí. (Kamrin, 1997; IUPAC, 2011).

Rozpustnost při 20–30 ºC (mg.l^{-1})		**Příklad**
Nerozpustné	< 1	quinoxyfen.
Mírně rozpustné	1–100	azoxystrobin, fludioxonil, mancozeb, penconazole, tebuconazole.
Rozpustné	100–10000	metalaxyl, propiconazole, pyrimethanil.
Velmi rozpustné	> 10000	
Poločas rozpadu fungicidů v půdě (d)		
Málo perzistentní	< 30	cyprodinil, mancozeb, metalaxyl, propiconazole, pyrimethanil, quinoxyfen.
Mírně perzistentní	30–100	azoxystrobin, fludioxonil, metalaxyl, propiconazole, pyrimethanil, quinoxyfen, tebuconazole.
Vysoce perzistentní	> 100	fludioxonil, penconazole, pyrimethanil, quinoxyfen, tebuconazole.
Mobilita fungicidů dle klasifikace SSLRC[a] (K_{oc} ml.g^{-1})		
Velmi mobilní	< 15	
Mobilní	15–74	metalaxyl.
Mírně mobilní	75–499	metalaxyl, pyrimethanil.
Nepatrně mobilní	500–4000	azoxystrobin, mancozeb, penconazole, propiconazole, pyrimethanil, tebuconazole.
Nemobilní	> 4000	fludioxonil, quinoxyfen.

[a] Soil Survey and Land Research Centre, Cranfield University, UK

Obrázek 1. Hlavní faktory ovlivňující chování fungicidů v životním prostředí Wheeler (2002).



2.3.2 Vlastnosti půdy ovlivňující sorpci fungicidů

Fungicidy jsou v půdě sorbovány na půdní částice. Do jaké míry se fungicidy v půdě sorbují, ovlivňuje především: obsah a složení organické hmoty, obsah jílových minerálů a oxyhydroxidů, velikost půdních částic, pH, atd. (Iglesias-Jiménez et al., 1997; Gevao et al., 2000; Clausen a Fabricius, 2001; Fernandes et al., 2006; Pateiro-Moure et al., 2009).

Obecně mezi nejdůležitější faktory ovlivňující sorpci organických fungicidů patří obsah organické hmoty v půdě (Riise et al., 2001; Berenzen et al., 2005; Fernandes et al., 2006; Rodríguez-Cruz et al., 2006) a kvalita a složení organické hmoty (Fernandes et al., 2006). Pokud humusové látky obsahují vyšší množství rozpustné organické hmoty, sorpce organických fungicidů může být nižší díky kompetici rozpustné organické hmoty a fungicidu o nespecifická sorpční místa (Fernandes et al., 2006). Andrades et al. (2001) a Monkiedje a Spiteller (2002) zdůrazňují, že také obsah jílových minerálů značně ovlivňuje retenci fungicidů v půdě a sorpce fungicidů při nízkém obsahu půdní organické hmoty je převážně ovlivněna půdními minerály (Sukop a Cogger, 1992; Sharma a Awasthi, 1997) a obsahem Fe, Mn-oxyhydroxidů v půdě (Thorstensen et al., 2001). Důležitou roli přitom hraje charakter minerálů (Singh, 2005). Z toho vyplývá, že sorpci fungicidů ovlivňuje hlavně složení půdy a kvalita jednotlivých půdních komponent. Lze tedy usuzovat, že půdy s nízkým obsahem jílu či organické hmoty by měly být obohaceny přídavkem těchto složek, čímž by bylo zabráněno proplavování fungicidů do spodních vod (Komárek et al., 2010).

2.3.3 Sorpce tebuconazolu v půdě

Sorpce a stejně tak i degradace tebuconazolu v půdě je převážně ovlivněna obsahem půdní organické hmoty (Berenzen et al., 2005). Hodnoty K_{oc} se pohybují v rozmezí 803−1251 l.kg^{-1} v závislosti na složení půdy. Tyto hodnoty poukazují na vysokou afinitu tebuconazolu k organickému uhlíku. Velmi důležitý je ale i obsah jílových částic. Hodnoty K_d se pohybují v rozmezí 7,7 (prach) až 12,4−16,4 l.kg^{-1} (písčitohlinitá půda), což indikuje, že půdy s vyšším obsahem jílových částic mají větší schopnost sorbovat tebuconazole (FAO, 1994; Bending et al., 2007). Jeho poločas rozpadu se pohybuje mezi 55,8–365 dny (IUPAC, 2011) v závislosti na množství aplikovaného fungicidu a půdním typu. Množství tebuconazolu adsorbovaného na půdu se pohybuje mezi 28%−74% (FAO, 1994). Dalším faktorem, který přispívá k vyšší sorpci tebuconazolu, je vegetační pokryv. Distribuční koeficient tebuconazolu na půdách s vegetačním pokryvem byl až 4krát větší, kvůli vyššímu množství organické hmoty ve svrchním půdním horizontu (Dousset et al., 2010). Tebuconazole je detekován hlavně v horních vrstvách půdy (0−15 cm), a proto by nemělo docházet k jeho průniku do spodních vod (FAO, 1994). I přesto byly naměřeny koncentrace tebuconazolu v povrchových vodách v Braunschweigu (Německo), které dosahovaly hodnot až 9,1 $\mu g.l^{-1}$ při aplikovaném množství fungicidu 250 $g.ha^{-1}$ (Berenzen et al., 2005). Uvážíme-li, že fungicidy mohou být akumulovány a adsorbovány na půdní částice, lze předpokládat, že následkem toho může docházet k výraznému ovlivňování půdních organismů a ekosystému. Po aplikaci tebuconazolu byl v půdách s nízkým obsahem půdní organické hmoty sledován pokles aktivity enzymu dehydrogenázy (Bending et al., 2007). Podobné výsledky uvádějí i Cycoń et al. (2006) a poukazují na negativní působení tebuconazolu na nitrifikační bakterie a bakterie fixující vzdušný dusík.

2.4 Degradace organických fungicidů v půdě

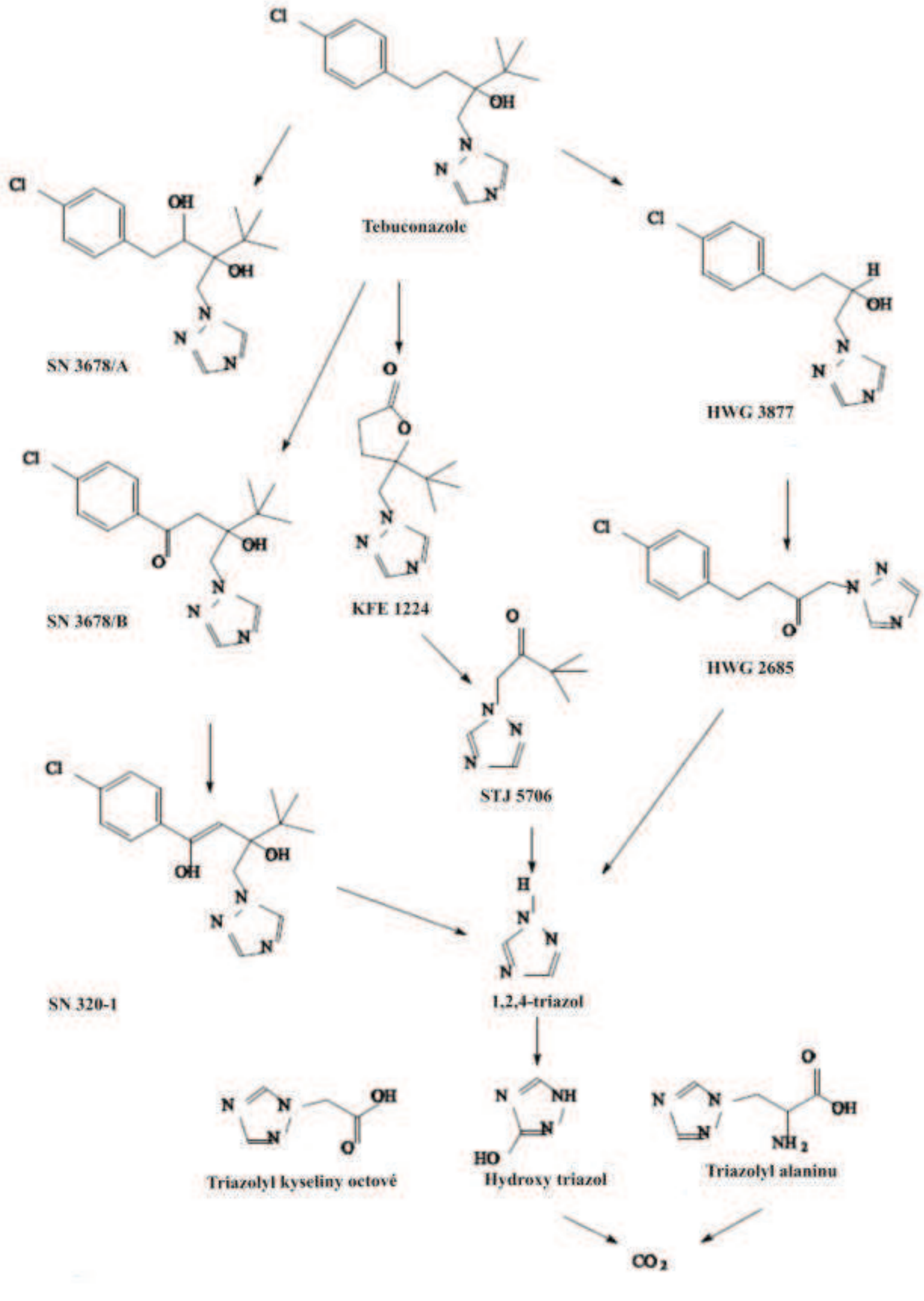
Stejně jako sorpce je i degradace fungicidů ovlivněna jejich fyzikálně-chemickými vlastnostmi, půdními vlastnostmi, vegetačním pokryvem půdy či mikrobiálním aktivitou v půdě. Vzhledem k tomu, že fungicidy patří do mnoha chemických skupin, je velmi těžké popsat obecné principy jejich degradace. Proto bude tato část zaměřena pouze na degradaci tebuconazolu v půdě. Degradace tebuconazolu v půdě je znázorněna na obrázku 2. Podle FAO (1994) většinu reziduí v půdě tvořil tebuconazole, 32% produktů rozkladu oxid uhličitý a pouze 0,8−3,5% bylo tvořeno ostatními produkty (v malém množství byl tvořen 1,2,4-triazol, který byl dále také degradován na oxid uhličitý). FAO (1994) uvádí, že průměrný poločas rozpadu tebuconazolu je v polních podmínkách 100 dní. Jak již bylo zmíněno dříve, poločas rozpadu záleží na fyzikálně-chemických vlastnostech pesticidu a půdních podmínkách. Vyšší vlhkost a teplota půdy v kombinaci s nízkým obsahem organického uhlíku může vést k rychlejší degradaci tebuconazolu. Díky nízkému obsahu organického uhlíku dochází k menší sorpci a tebuconazole se tak stává lépe biodostupným. Poločas rozpadu byl za těchto podmínek až 7krát nižší (Strickland et al., 2004), než byly hodnoty uvedené např. v IUPAC (2011). Další faktor, který ovlivňuje poločas rozpadu je vegetační pokryv. Vegetace přispívá k větší mikrobiální diverzitě v půdě a tím podporuje rychlejší degradaci tebuconazolu (FAO, 1994).

Přestože je tebuconazole používán proti chorobám způsobeným houbami, právě houby a jiné mikroorganismy mohou přispívat k jeho přeměnám a degradaci. Obanda a Shupe (2009) uvádějí, že izoláty z hub *Meruliporia incrassata, Chaetomium globosum* a *Trichoderma harzianum* mohou degradovat tebuconazole v kapalném médiu během 21 dní. Tebuconazole v pevném médiu (dřevě) byl velmi dobře degradován houbami *Trametes* *versicolor* a *Fomitopsis palustris*. Jiné houby (*Phanerochaete chrysosporium*, *Laetiporus sulphureus*, *Coniophora puteana*, *Trichoderma harzianum*) byly schopné na této matrici růst (Woo et al., 2010) a lze tedy předpokládat, že po delší expozici tebuconazolu by ho byly taktéž schopny degradovat.

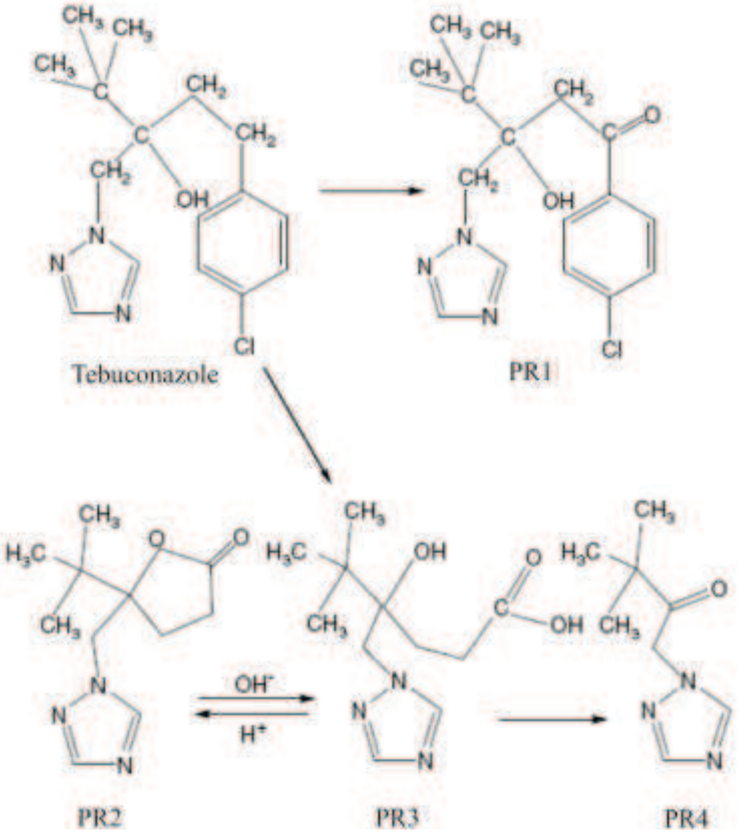
Hlavní krok detoxifikace je štěpení triazolového kruhu, který je odpovědný za fungicidní charakter látek. Jakmile tedy dojde ke zničení triazolového kruhu, molekula ztrácí svoji schopnost inhibovat růst organismů. V důsledku toho se pak mikroorganismy mohou opět úspěšně vyvíjet. Kromě toho dochází k oxidačním reakcím na *terc*-butyl skupině a oxidační produkty jsou dále metabolizovány acetylací. Vzniklé produkty rozkladu jsou netoxické (Obanda et al., 2008).

Strickland et al. (2004) uvádějí, že rozkladem tebuconazolu v půdě vznikají 4 hlavní produkty rozkladu - PR1, PR2 (γ–lakton), PR3 (γ–hydroxy kyselina-forma γ–laktonu) a PR4 (Obrázek 3), kde PR2 a PR4 odpovídají produktům rozkladu tebuconazolu KFE 1224 a STJ 5706 dle FAO (1994). Z jejich studie vyplývá, že při degradaci tebuconazolu byl v nejvyšší míře tvořen PR3 (tvořil téměř 50% produktů rozkladu) a následně PR2 (tvořil 15% všech produktů rozkladu). Další dva produkty rozkladu PR1 s PR4 byly tvořeny minimálně (méně než 2% všech produktů rozkladu). Podobné výsledky byly zjištěny ve studii Potter et al. (2005), kdy byly detekovány produkty rozkladu PR1, PR2 (γ–lakton) a PR4 (obrázek 3). PR3 nebyl detekován pravděpodobně z důvodu jeho rychlé přeměny na PR2. PR2 v tomto případě tvořil 92–97% produktů rozkladu tebuconazolu, zatímco PR1 a PR4 pouze 1%.

Obrázek 2. Degradace tebuconazolu v půdě (FAO, 1994).



Obrázek 3. Hlavní produkty rozkladu tebuconazolu v půdě (Strickland et al., 2004).



2.5 Interakce organických pesticidů a fungicidů na bázi mědi

2.5.1 Sorpce mědi v půdě

Pozaďové obsahy Cu v nekontaminovaných půdách jsou ovlivněny mateční horninou, ze které byly půdy tvořeny a dosahují průměrných hodnot 30 mg.kg^{-1} (Adriano, 2001). Rozpustnost a mobilita mědi je hlavně ovlivněna hodnotou pH půdy; nejdostupnější je měď při hodnotách pH 6 (Adriano, 2001; Celardin et al., 2004; Boudesocque et al., 2007). V kyselých půdách tedy Cu může snadno migrovat půdním profilem a způsobit tak znečištění podzemních vod (Nóvoa-Muñoz et al., 2007). Měď je v půdách adsorbována hlavně na půdní organickou hmotu a hydroxidy železa a manganu, v menší míře pak na jílové minerály (Parat et al., 2002; Bradl, 2004). Nicméně půdní organická hmota může ovlivnit mobilitu Cu dvěma způsoby. Zatímco nerozpustná půdní organická hmota působí jako sorbent Cu, rozpustná půdní organická hmota s Cu komplexuje a tím zvyšuje její rozpustnost zvláště při zásaditém pH (cca 7,5) půdy (Arias et al., 2006; Fernández-Calviño et al., 2008; Martínez-Villegas a Martínez, 2008). Dalším důležitým faktorem, který ovlivňuje mobilitu a biodostupnost Cu v půdách jsou půdní uhličitany. Aktivita Cu ve vápenatých půdách je do značné míry kontrolována precipitací $CuCO_3$ (Ponizovsky et al., 2007). Důležitým retenčním mechanismem je srážení nově se tvořících Cu fází mezi které patří: $Cu(OH)_2$, $CuCO_3/Cu_2(OH)_2CO_3$, CuO. Pokud se v půdách nachází sírany (např. z aplikací Bordeauxské jíchy), dochází ke srážení a tvorbě mnoha hydroxysulfátů (Ma et al., 2006; Ponizovsky et al., 2007; Komárek et al., 2009). Aplikací fosfátů do půdy (např. ve formě hnojiv) dochází ke tvorbě nových fází, což následně může snižovat mobilitu rizikových kovů v půdě (Cao et al., 2003; Pérez-Novo et al., 2009).

2.5.2 Interakce mezi organickými pesticidy a mědí

Jak již bylo uvedeno dříve, prvotně byly proti houbovým chorobám používány fungicidy na bázi Cu. V důsledku toho poté docházelo k akumulaci Cu v horních půdních horizontech (Arias et al., 2004). Právě obsah Cu v půdě ale může významně ovlivňovat adsorpci organických fungicidů. Pateiro-Moure et al. (2007) se zabývali studiem interakcí mezi Cu a organickými herbicidy (paraquat, diquat, difenzoquat) a zjistili, že při zvýšených koncentracích Cu může docházet k vytěsňování difenzoquatu z adsorpčních míst. Arias et al. (2006) uvádějí, že přítomnost Cu nijak neovlivnila sorpci organického fungicidu metalaxyl. Na druhou stranu ale přídavek Cu zvýšil sorpci fungicidu penconazole díky tvorbě komplexů Cu-penconazole, které měly vyšší afinitu k půdním koloidům. Podobně byly tvořeny komplexy i při interakcích Cu s herbicidem glyfosát (zvláště v kyselých půdách) (Morillo et al., 2000) a dále při interakcích Cu s fungicidem tebuconazole (Zhang and Wu, 2005; Evans et al., 2007; Jaklová Dytrtová et al., 2011). Jak již bylo zmíněno dříve, interakce Cu s organickými pesticidy ovlivňují jejich toxicitu a chování v půdě. Jacobson et al. (2005) uvádějí, že zvýšené koncentrace Cu významně ovlivnily změny ve složení mikrobiální populace, a tím transport diuronu v půdě. Z tohoto důvodu je velmi důležité sledovat jednotlivé interakce mezi anorganickými a organickými fungicidy. Díky detailnímu studiu těchto látek a jejich chování v půdě je možné předpovídat jejich chování v půdním profilu a popř. tak omezit negativní dopady na životní prostředí.

3. Hypotézy a cíle práce

Cílem této práce je posouzení vlivu odlišných půdních podmínek (typ půd, zastoupení půdních minerálů, obsah půdní organické hmoty, hodnota pH, přítomnost Cu) na retenci organického fungicidu tebuconazole v půdách. Všechny experimenty jsou prováděny pomocí rovnovážných vsádkových („batch“) experimentů.

Cíle práce jsou formulovány na základě těchto hypotéz:

1) **Hypotéza:** Odlišné složení půd (obsah a složení organické hmoty, zastoupení půdních minerálů, popř. hodnota pH) může výrazně ovlivnit sorpci tebuconazolu.

Cíl: Studium retence tebuconazolu v kontrastních typech půd (kambizem, regozem, rendzina) – vsádkové („batch“) experimenty.

2) **Hypotéza:** Odlišné složení a vlastnosti vybraných minerálů a humínových kyselin (např. specifický povrch) mohou významně ovlivnit sorpci tebuconazolu. Rozdílné pH hodnoty mohou také výrazně ovlivnit sorpci tebuconazolu.

Cíl: Studium retence tebuconazolu na vybraných synteticky připravených půdních minerálech (birnessit, ferrihydrit, goethit, kalcit), illitu a humínových kyselinách v závislosti na pH.

3) **Hypotéza:** Přítomnost Cu či tvorba Cu-azol komplexů, mohou ovlivňovat adsorpci tebuconazolu na půdní částice či minerály, popř. kompletně změnit adsorpční mechanismy.

Cíl: Studium vlivu přítomnosti Cu (popř. tvorby Cu-tebuconazole komplexů) na adsorpci tebuconazolu – vsádkové („batch“) experimenty.

4. Materiál a metody

4.1 Sorpce tebuconazolu na synteticky připravené minerály a humínové kyseliny

Ferrihydrit a goethit byly připraveny podle metody popsané v publikaci Schwertmann a Cornell (2000), birnessit dle McKenzie (1971) a kalcit dle Vaněk et al. (2010). Illit (standard IMt-1) byl zakoupen z The Source Clays Repository (Purdue University, Indiana, USA). Humínové kyseliny stejně jako ostatní chemikálie byly zakoupeny od Sigma-Aldrich (USA). Syntetické minerální fáze byly ověřeny pomocí XRD a voltametrie mikročástic. Veškeré chemikálie byly čistoty p.a. Hlavní charakteristiky vybraných minerálů jsou uvedeny v tabulce 3.

Tabulka 3. Hlavní charakteristiky vybraných minerálů.

Minerál	**Vzorec**	**Specifický povrch ($m^2.g^{-1}$)**
Birnessit	$K_4Mn_{14}O_{27}.9H_2O$	35,4–36,2 [a,b]
Kalcit	$CaCO_3$	4,8–5,8 [c,d]
Ferrihydrit	$Fe_5HO_8 \cdot 4H_2O$	200–320 [e]
Goethit	α-FeOOH	20 [e]
Illit	$(K,H_3O)Al_2(Si,Al)_4O_{10}(OH)_2$	11 [f]

[a] Cheney et al. (2008); [b] O'Reilly a Hochella (2003); [c] Mihajlović et al. (2009); [d] Clausen et al. (2001); [e] Schwertmann a Cornell (2000); [f] Wei et al. (2006)

Tebuconazole byl použit ve formě standardu zakoupeného v Institute of Industrial Organic Chemistry, Varšava (Polsko) a ve formě komerčně dostupného výrobku Horizon 250 EW vyrobeného v Bayer CropScience (Německo). Všechny ostatní chemikálie (methanol, ethylacetát) byly čistoty Chromapur G a byly zakoupeny v Chromservisu (Česká Republika).

Tebuconazole ve formě standardu byl rozpuštěn v methanolu ($1g.l^{-1}$) a uchováván v chladu a tmě. Z tohoto roztoku byly dále připraveny roztoky tebuconazolu o koncentracích (50–175 $mg.l^{-1}$) v 0,01 M $CaCl_2$. 0,01 M $CaCl_2$ byl zvolen jako pozaďový elektrolyt vzhledem k jeho širokému použití v jiných studiích (Clausen et al., 2001; Jia et al., 2007; Baglieri et al., 2009) a dále byl tento elektrolyt doporučen při vsádkových („batch“) experimentech směrnicí OECD 106 (2000). Methanol byl poté odstraněn pomocí rotační vakuové odparky (Rotavapor R-215, Büchi, Švýcarsko).

Roztoky tebuconazolu z komerčně dostupného prostředku Horizon 250 EW o koncentracích (50–175 $mg.l^{-1}$) byly připraveny rozpuštěním daného množství přípravku v 0,01 M $CaCl_2$.

Půdní minerály a humínové kyseliny byly ponechány 24 hodin k ekvilibraci v 0,01 M $CaCl_2$. Dále následovala úprava hodnot pH suspenzí minerálů přídavkem HCl či $Ca(OH)_2$ na hodnoty pH 4, 5,5 a 7. Hodnoty pH suspenzí kalcitu (pH 7,3) a humínových kyselin (pH 3,3) nebyly upravovány vzhledem k jejich vysoké pufrační kapacitě.

K 40 ml suspenze minerálů/humínových kyselin bylo přidáno 10 ml roztoku tebuconazolu o požadovaných koncentracích, vzorky byly ponechány 24 hodin na třepačce (GFL 3017 orbital shaker, Německo) a poté centrifugovány 10 min při 7000 ot/min (Hettich Universal 30 RF centrifuge, Německo). Odebraný supernatant (45 ml) byl extrahován 1 hod na třepačce organickým rozpouštědlem ethylacetát (20 ml), který byl následně odebrán a analyzován pomocí GC/MS (Agilent Technologies 6890N/5975, USA). Všechny vzorky byly provedeny ve třech opakováních a ve tmě.

Chromatografická separace byla provedena na koloně RTX-50 (délka 30 m, průměr 250 μm, tloušťka filmu 0,25 μm). Jako nosný plyn bylo použito helium čistoty 5,0 s průtokem 2,5 $ml.min^{-1}$. Nástřik (1μl) byl proveden v metodě splitless při teplotě 280 ºC. Hmotnostní spektrometrická detekce byla provedena ve SCAN režimu. Pro kalibraci byly použity roztoky tebuconazolu připravené ze standardního materiálu.

4.2 Sorpce tebuconazolu na kontrastní typy půd

Byly vybrány a odebrány tři kontrastní typy půd (kambizem, regozem a rendzina). Hlavní půdní charakteristiky daných půd jsou uvedeny v tabulce 4.

Roztoky tebuconazolu byly připraveny stejně jako v předešlém experimentu.

K 5 g zeminy bylo přidáno 50 ml roztoku tebuconazolu o požadovaných koncentracích. Vzorky byly ponechány 48 hodin na třepačce a poté centrifugovány 10 min při 7000 ot/min. Odebraný supernatant (45 ml) byl extrahován 1 hod na třepačce organickým rozpouštědlem ethylacetát (20 ml), který byl následně odebrán a analyzován pomocí GC/MS (Agilent Technologies 6890N/5975, USA). Všechny vzorky byly provedeny ve třech opakováních a ve tmě.

Tabulka 4. Hlavní charakteristiky kontrastních typů půd.

	Rendzina	**Regozem**	**Kambizem**
Zastoupení půdních částic (%)			
Jíl	0,4	1,3	3,2
Prach	30,6	6,4	32,6
Písek	69,0	92,3	64,2
pH $_{\mathrm{H2O}}$	7,4	6,7	5,6
pH $_{\mathrm{KCl}}$	7,0	6,4	4,7
pH $_{\mathrm{ZPC}}$	7,7	6,5	5,1
KVK [a] **(cmol.kg**$^{-1}$**)**	28,3	10,1	31,5
TOC [b] **(%)**	3,36	1,16	3,78
TIC [c] **(g.kg**$^{-1}$**)**	3,10	-	-
Obsah amorfních a slabě krystalických oxidů a hydroxidů Fe, Mn a Al (g.kg^{-1}**)**			
Fe	2,23	1,91	1,63
Al	1,55	1,14	2,55
Mn	0,30	0,10	0,69
Identifikované minerály			
	křemen kalcit albit ortoklas illit kaolinit	křemen muskovit albite ortoklas amfibol klinopyroxen illit klinochlor	křemen muskovit albite ortoklas illit klinochlor
Humínové látky (%)			
Humínové kyseliny	74,1	59,2	55,0
Fulvokyseliny	3,7	8,5	8,3
Hydrofilní kyseliny	15,4	15,9	26,3
Hydrofobní neutrální organická hmota	6,8	16,4	10,4

\- pod mezí detekce

[a] kationtová výměnná kapacita

[b] celkový obsah organického uhlíku

[c] celkový obsah anorganického uhlíku

4.3 Sorpce tebuconazolu a Cu na kontrastní typy půd, humínové kyseliny a synteticky připravený minerál ferrihydrit

Roztoky tebuconazolu o různých koncentracích byly připraveny stejně jako v předešlých experimentech. Zásobní roztoky Cu (v 0,01 M $CaCl_2$) byly připraveny tak, aby látkové množství Cu a tebuconazolu bylo stejné, tedy byl zachován poměr tebuconazole : Cu (Teb : Cu) 1 : 1. Dále byly připraveny zásobní roztoky s poměrem Teb : Cu 1 : 4 a 4 : 1.

Sorpční experiment byl prováděn stejně jako v předchozích případech a za identických podmínek. Jediným rozdílem je přídavek zásobního roztoku mědi do suspenze ferrihydritu, humínových kyselin či půd. Obsah mědi byl analyzován za využití optické emisní spektrometrie s indukčně vázaným plazmatem (ICP-OES) na přístroji Varian VistaPro (Varian, Austrálie).

Množství sorbovaného tebuconazolu na minerály, humínové kyseliny či půdy bylo vypočítáno z rozdílu počáteční koncentrace tebuconazolu a rovnovážné koncentrace tebuconazolu po jeho sorpci (vztažené na objem kapalné fáze a množství dané matrice).

4.4 Sestrojení adsorpčních izoterem

Adsorpční izotermy byly sestrojeny pomocí následujících rovnic:

(i) Langmuirova izoterma sestrojená pomocí následující rovnice:

$$S = S_{\max} \times \frac{KC}{1 + KC} \tag{1}$$

(ii) Sigmoidální Langmuirova izoterma sestrojená pomocí následující rovnice:

$$S = S_{\max} \times \frac{KC}{1 + KC + \frac{s}{C}} \tag{2}$$

(iii) Freundlichova izoterma sestrojená pomocí následující rovnice:

$$S = K_f \times C^n \tag{3}$$

kde S je koncentrace sorbovaného tebuconazolu (µmol.g^{-1}), S_{max} je maximální koncentrace sorbovaného tebuconazolu (µmol.g^{-1}), C je rovnovážná koncentrace tebuconazolu (µmol.l^{-1}), K je Langmuirův adsorpční koeficient (l.µmol^{-1}), s je konstanta vztahující se k omezení adsorpce (g.l^{-1}), konstanty Kf (l.g^{-1}) a n (bezrozměrná) charakterizují kapacitu a intenzitu adsorpce.

Langmuirovy a Freundlichovy izotermy byly sestrojeny podle programu vyvinutého k získání parametrů izoterem (pomocí součtu nejmenších čtverců) a modelu účinnosti (Bolster a Hornberger, 2007). Sigmoidální Langmuirovy izotermy byly sestrojeny pomocí metody součtu nejmenších čtverců v programu Mathematica 7 (Wolfram Research, Inc., 2008).

5. Výsledky a diskuze

Organické a anorganické prostředky k ochraně zemědělských plodin patří mezi látky, které budou stále hojně využívány. Jak již bylo zmíněno dříve, mezi často používané látky k ochraně rostlin, osiv, ale i materiálů patří fungicidy ze skupiny triazolů. V České Republice, ale i celosvětově výrazně vstoupá spotřeba fungicidů obsahující účinnou látku tebuconazole (ČR: 28,6 t/2003; 105,7 t/2010) (SRS; Státní rostlinolékařská správa). I přesto, že je tebuconazole převážně aplikován na rostliny, dostává se do půdy právě při aplikacích nebo smyvem z rostlin. Jedná se o látku, která se velmi dobře sorbuje na půdní organickou hmotu, ale i ostatní půdní komponenty a může se tak akumulovat v prostředí a negativně působit na půdní ekosystém. Dosavadní výzkum byl především zaměřen na sledování degradace tebuconazolu či jeho vliv na rostliny a půdní mikroorganismy. V předložené disertační práci je sledována sorpce tebuconazolu na odlišné typy půd, jednotlivé půdní komponenty a interakce této účinné látky s Cu, která byla jak v minulosti, tak i dnes, běžnou součástí fungicidních přípravků. Následkem dlouhodobého používání fungicidů na bázi Cu, dochází k akumulaci Cu ve svrchních půdních horizontech, což nadále ovlivňuje adsorpci organických fungicidů (Arias et al., 2006; Komárek et al., 2010). V případě azolových fungicidů dochází k tvorbě Cu-azol komplexů, které ovlivňují sorpční schopnosti obou látek. Z obdržených výsledků je možné predikovat chování tebuconazolu v půdním prostředí a jeho schopnost interakce s anorganickými fungicidy obsahující Cu, popř. tak omezit negativní vliv na životní prostředí.

Pro sledování sorpce tebuconazolu byly použity půdy s odlišnými vlastnostmi a složením (kambizem, regozem a rendzina). Mezi nejdůležitější faktory ovlivňující sorpci tebuconazolu patří rozdílný obsah půdní organické hmoty, její kvalita, složení a obsah půdních minerálů a další. Pro podrobnější studii sorpce tebuconazolu na jednotlivé půdní komponenty byly použity synteticky připravené půdní minerály (birnessit, ferrihydrit, goethit a kalcit), illit a humínové kyseliny (představující půdní organickou hmotu). Minerály byly vybrány na základě jejich odlišných vlastností, a také proto, že jsou běžnými složkami půd a tvoří významnou složku půdního sorpčního komplexu.

Tebuconazole byl použit ve formě standardní látky, ale také ve formě komerčně dostupného přípravku Horizon 250EW. Většina studií zabývající se chováním fungicidů v životním prostředí, provádí experimenty s čistými chemikáliemi i přesto, že v zemědělské praxi nejsou v této formě používány. Aditiva přítomná v komerčně dostupných prostředcích mohou významně ovlivnit chování fungicidů v prostředí. Jejich funkcí je především usnadnění aplikace, zvýšení efektivnosti či zabránění rekrystalizace účinné látky. Horizon 250EW obsahuje až 75% organických aditiv (převážně N,N-dimethyldekanamid). Jak již bylo zmíněno dříve, tebuconazole vykazuje vysokou afinitu k organickým látkám, a proto lze předpokládat, že právě organická aditiva mohou ovlivnit jeho sorpci a chování v půdním profilu v porovnání s čistou chemikálií.

5.1 Sorpční experimenty s tebuconazolem ve formě standardní látky

5.1.1 Sorpce tebuconazolu ve formě standardní látky na synteticky připravené minerály a humínové kyseliny

Jedním z našich cílů bylo objasnit, zda odlišné složení a vlastnosti vybraných minerálů a humínových kyselin mohou významně ovlivnit sorpci tebuconazolu (viz Čadková et al., 2012). Adsorpční izotermy byly nejlépe popsány sigmoidálními Langmuirovými izotermami (ferrihydrit, goethit a birnessit) či Langmuirovými izotermami (illit, humínové kyseliny). Jak již bylo uvedeno dříve, suspense minerálů (kromě kalcitu) byly upravovány na hodnoty pH 4, 5,5 a 7, aby byl následně porovnán vliv rozdílných hodnot pH na sorpci tebuconazolu. Z výsledků vyplývá, že odlišné hodnoty pH neměly vliv na sorpci tebuconazolu ve formě čisté chemikálie. Nejvyšší sorpční schopnost vykazoval ferrihydrit (28 μmol.g^{-1}), dále illit (9 μmol.g^{-1}) a sorpce tebuconazolu byla téměř zanedbatelná pro goethit a birnessit. Tyto výsledky jsou v souladu s jinými studiemi, které potvrzují nízkou adsorpci neiontových pesticidů na ferrihydrit a goethit (Clausen a Fabricius, 2001). Kalcit má malý specifický povrch a při hodnotách pH pohybujících se okolo hodnoty 7, na jeho povrchu převládají místa bez náboje např. $-\mathrm{CaOH}^0$ (Geffroy et al., 1999). Tyto skutečnosti pravděpodobně přispěly k tomu, že sorpce tebuconazolu na kalcit nebyla zaznamenána. Na rozdíl od minerálů byla sorpce tebuconazolu na humínové kyseliny výrazně vyšší (89 μmol.g^{-1}). Humínové kyseliny, které v tomto případě zastupují půdní organickou hmotu, jsou hlavním faktorem ovlivňujícím sorpci tebuconazolu, což potvrzuje obecné poznatky, že půdní organická hmota sorbuje organické pesticidy daleko více než ostatní půdní komponenty (minerály včetně oxidů) (Baglieri et al., 2009; Iglesias et al., 2009). Nicméně Singh (2005) poukazuje na to, že obsah půdní organické hmoty není nejdůležitějším faktorem a vyzdvihuje důležitou roli jílových minerálů. Naše data popisující sorpci tebuconazolu na illit s těmito závěry ale příliš nekorespondují.

5.1.2 Sorpce tebuconazolu ve formě standardní látky na kontrastní typy půd

Dalším cílem bylo zjistit, zda odlišné složení půd (obsah a složení organické hmoty, zastoupení půdních minerálů, popř. hodnota pH) může výrazně ovlivnit sorpci tebuconazolu (viz Čadková et al., in press). Adsorpční izotermy byly nejlépe popsány Langmuirovými izotermami. Z výsledků vyplývá, že trend sorpce tebuconazolu na vybrané půdy byl následující: kambizem > regozem > rendzina. Kambizem obsahuje nejvyšší obsah půdní organické hmoty, a na základě našich výsledků byl potvrzen předpoklad, že se na ni bude tebuconazole sorbovat v nejvyšší míře. Rozdílně tomu bylo ale u dalších typů půd. Ačkoli rendzina obsahuje více půdní organické hmoty než regozem, byla u ní zaznamenána nejnižší sorpční schopnost. Proto pravděpodobně nejen obsah, ale i kvalita půdní organické hmoty (ve smyslu obsahu hydrofobní neutrální organické hmoty a obsahu humínových, hydrofilních a fulvokyselin) je důležitým faktorem ovlivňujícím sorpci látek. Regozem obsahuje vysoký podíl hydrofobní neutrální organické hmoty, která podporuje sorpci hydrofobních organických látek - mezi které tebuconazole patří (Kaiser et al., 2001), což mohlo přispět k vyšší sorpční schopnosti této půdy. Mnoho autorů uvádí také vysokou schopnost sorpce humínových kyselin v porovnání s fulvokyselinami (Sparks, 2003; Hiller et al., 2009; Iglesias et al., 2009). Kambizem obsahuje nejvyšší množství humínových kyselin. Také ale obsahuje, stejně jako regozem, vysoký podíl fulvokyselin, které obecně vykazují nižší afinitu k nepolárním organickým látkám (Hiller et al., 2009). I přesto, že je tebuconazole nepolární a kambizem a regozem obsahují vysoký podíl fulvokyselin, obě půdy vykazují vysokou schopnost sorbovat tebuconazole. Důvodem je pravděpodobně odlišný původ a složení fulvokyselin. Sorpční schopnost humínových kyselin a fulvokyselin závisí hlavně na jejich původu, stáří, ale i na okolních vlastnostech prostředí např. vegetaci, teplotě. Sorpce dané látky se tak může až řádově lišit právě v závislosti na původu humínových kyselin či fulvokyselin (Niederer et al., 2007). Tyto poznatky vysvětlují nízkou sorpční schopnost rendziny, i přestože obsahuje relativně vysoký podíl humínových kyselin. Dalším důvodem pro vyšší sorpci regozemě může být i vysoký obsah jílových minerálů a jejich složení. Regozem a stejně tak i kambizem obsahují klinochlor, který patří do skupiny chloridů, u nichž byla zjištěna schopnost sorbovat organické látky (Koutsopoulou et al., 2010), což v konečném důsledku přispělo k vyšší sorpční schopnosti regozemě. Singh (2005) shodně ve své studii uvádí nižší sorpční schopnost půd, i přestože obsahují vyšší obsah půdní organické hmoty. Z toho lze usuzovat, že také množství a složení jílových minerálů může hrát významnou roli při sorpci tebuconazolu.

5.1.3 Sorpce tebuconazolu ve formě standardní látky na kontrastní typy půd, humínové kyseliny a synteticky připravený minerál ferrihydrit v přítomnosti Cu

Posledním cílem bylo objasnit, zda přítomnost Cu či tvorba Cu-azol komplexů, mohou ovlivňovat sorpci tebuconazolu na půdní částice či minerály. Po vyhodnocení výsledků byl zjištěn následující trend sorpce tebuconazolu (v přítomnosti Cu) na kambizem a humínové kyseliny (matrice obsahující nejvyšší množství organické hmoty): vzorky bez přídavku Cu > a dále poměry Teb : Cu 1 : 4 ≥ 1 : 1 > 4 : 1. Vysoká sorpce tebuconazolu u vzorků, které neobsahovaly Cu je pravděpodobně způsobena kompeticí Cu a tebuconazolu o adsorpční místa (Xu et al., 2005). Je známo, že tebuconazole je schopen tvořit komplexy s Cu (Zhang a Wu, 2005; Evans et al., 2007; Jaklová Dytrtová et al., 2011), následkem čehož může docházet k vyšší sorpci této látky. Tento fakt potvrzují získané výsledky, kdy vyšší poměr Cu : Teb přispěl k vyšší sorpci tebuconazolu. Tebuconazole ve formě standardní látky neobsahuje aditiva, která by podporovala jeho sorpci, proto je tebuconazole pravděpodobně prvotně sorbován na půdní organickou hmotu a teprve poté na další půdní komponenty. Tuto domněnku podporují výsledky získané při experimentu sorpce tebuconazolu na ferrihydrit, kde množství sorbovaného tebuconazolu ve vzorcích, které neobsahovaly Cu, bylo zanedbatelné. Naopak, vyšší sorpce tebuconazolu na ferrihydrit byla pozorována, pokud byla v roztoku přítomna Cu, což opět poukazuje na pozitivní vliv komplexace na sorpční schopnost tebuconazolu. Podobný trend byl zaznamenán i u regozemě, která obsahuje nejnižší množství půdní organické hmoty, ale relativně vysoký obsah amorfních a slabě krystalických oxidů Fe. Jako v případě ferrihydritu, vyšší koncentrace Cu pozitivně ovlivnily sorpci tebuconazolu na regozem důsledkem tvorby Teb-Cu komplexů. Nižší koncentrace Cu (Teb : Cu 4 : 1 a 1 : 1) pravděpodobně nejsou dostatečné k vytvoření těchto komplexů a sorpce tebuconazolu je pak nižší než u vzorků, které neobsahovaly přídavek Cu. Podobné výsledky byly pozorovány i v případě rendziny, která na rozdíl od regozemě obsahuje relativně vysoké množství půdní organické hmoty. K nejvyšší sorpci tebuconazolu docházelo při poměru Teb : Cu 1 : 4, a dále pak následovaly vzorky bez přídavku Cu ≈ 1 : 1 ≈ 4 : 1.

5.2 Sorpční experimenty s tebuconazolem ve formě komerčně dostupného přípravku

5.2.1 Sorpce tebuconazolu ve formě komerčně dostupného přípravku na synteticky připravené minerály a humínové kyseliny

Jak již bylo uvedeno dříve, jedním z cílů této práce bylo objasnit, jestli odlišné složení a vlastnosti vybraných minerálů či humínových kyselin mohou ovlivnit sorpci tebuconazolu ve formě komerčně dostupného prostředku (viz Čadková et al., 2012). Sorpce tebuconazolu ve formě komerčního přípravku na ferrihydrit, goethit, a birnessit byla významně větší než při použití standardní látky. Adsorpční izotermy těchto tří minerálů byly nejlépe popsány sigmoidální Langmuirovou izotermou. Tento tvar křivky je pravděpodobně výsledkem kooperativní sorpce organických aditiv přítomných v komerčním prostředku a tebuconazolu. Organická aditiva, např. surfaktanty, jsou nejdříve sorbovány na povrch minerálů a poté podporují a zvyšují sorpci dalších organických látek, např. fungicidů (Zhang et al., 2007). Nepolární organické látky (např. tebuconazole) vykazují nízkou afinitu k jílovým minerálům. Pokud je tedy povrch pokrytý organickými aditivy, jiné organické molekuly mohou být poté snáze adsorbovány (Limousin et al., 2007; Pose-Juan et al., 2010). Nejvyšší sorpční schopnost vykazoval ferrihydrit při hodnotě pH 4 (202 μmol.g^{-1}), přičemž se zvyšující hodnotou pH sorpce klesala (shodně tomu tak bylo i u goethitu a birnessitu). Stejný trend byl popsán i v další studii zabývající se organickými pesticidy (Clausen a Fabricius, 2001). Nicméně Jia et al. (2007) uvádějí sorpci jiných triazolových látek i při hodnotách pH 7. Sorpce tebuconazolu na goethit byla zaznamenána pouze při nízké hodnotě pH 4 (0,015 μmol.g^{-1}) a byla v porovnání s ferrihydritem o jeden řád nižší. Podobné výsledky byly publikovány i u iontových pesticidů (Clausen a Fabricius, 2001). Důvodem je pravděpodobně rozdílná hodnota specifického povrchu minerálů (200−320 m^2.g^{-1} ferrihydrit a 20 m^2.g^{-1} goethit) (Schwertmann a Cornell, 2000). Sorpce tebuconazolu na birnessit byla opět vyšší při nižších hodnotách pH (38 μmol.g^{-1}) a byla až 10krát vyšší v porovnání se sorpcí tebuconazolu ve formě standardní látky. Totéž platilo i pro illit, kdy sorpce tebuconazolu byla až 2krát vyšší (24 μmol.g^{-1}), pokud byl tebuconazole použit v komerční formě. Arias et al. (2006) shodně poukazují na to, že sorpce azolového fungicidu penconazole na illit byla po přídavku organických surfaktantů výrazně vyšší. Jako v předešlém experimentu nebyla zaznamenána sorpce tebuconazolu na kalcit. Sorpce tebuconazolu ve formě komerčního přípravku na humínové kyseliny byla opět mnohem vyšší v porovnání s vybranými minerály (s výjimkou ferrihydritu). Adsorpční izotermy popisující sorpci této látky na humínové kyseliny byly nejlépe popsány Freundlichovou izotermou. Zdánlivá nižší afinita tebuconazolu (v komerčním přípravku) k humínovým kyselinám (ve srovnání se standardní látkou) při nižších koncentracích byla pravděpodobně způsobena kompeticí tebuconazolu a organických aditiv přítomných v komerčním přípravku. Nicméně organická aditiva v komerčním prostředku nijak neovlivňovala míru sorpce tebuconazolu na humínové kyseliny. Jak již bylo zmíněno dříve, humínové kyseliny, které zastupují půdní organickou hmotu, jsou v porovnání s půdními minerály nejefektivnějším sorbentem pro organické fungicidy (Pose-Juan et al., 2010). Vyšší sorpce tebuconazolu na ferrihydrit (v porovnání s humínovými kyselinami) byla pravděpodobně způsobena jeho velkým specifickým povrchem, na který se mohou navázat organická aditiva, která následně podpoří sorpci tebuconazolu.

Rozdíl v sorpci tebuconazolu ve formě standardní látky a komerčního přípravku je způsobem přítomností aditiv (např. N,N-dimethyldekanamid). Tebuconazole vykazuje vysokou afinitu k organické hmotě a právě aditiva jsou téměř výhradně organického původu. Sanchez-Martin et al. (2006) uvádějí podobné výsledky. Porovnávali sorpční schopnost přírodních minerálů a minerálů upravených organickými surfaktanty. Sorpční schopnost přírodních minerálů byla mnohonásobně nižší v porovnání s modifikovanými minerály. Sorpce azolových fungicidů na takto upravených minerálech může být až 133krát vyšší právě kvůli vyššímu množství organického uhlíku, který surfaktanty obsahují (Rodríguez-Cruz et al., 2008). Nicméně množství adsorbovaných aditiv a dále též sledované látky, se liší v závislosti na typu či specifickém povrchu minerálů. Tento fakt vysvětluje sorpci tebuconazolu na námi vybrané minerály v pořadí: ferrihydrit > birnessit > goethit > illit > kalcit.

5.2.2 Sorpce tebuconazolu ve formě komerčně dostupného přípravku na kontrastní typy půd

V práci Čadková et al. (in press) zabývající se sorpcí tebuconazolu na kontrastní typy půd byla nejvyšší sorpce pozorována u kambizemě > rendziny > regozemě. Stejně jako v předešlém experimentu s tebuconazolem ve formě standardní látky je sorpce tohoto fungicidu převážně závislá na obsahu půdní organické hmoty. Kambizem obsahuje její nejvyšší množství v kombinaci s nejvyšším obsahem hydrofobní neutrální organické hmoty, která podporuje sorpci organických látek a nejvyšším obsahem jílových částic a oxyhydroxidů Mn. Přestože se obsah půdní organické hmoty u rendziny a regozemě značně liší, vykazují obě půdy podobné sorpční schopnosti, ačkoli vyšší obsah půdní organické hmoty rendziny vedl k její vyšší sorpci tebuconazolu. Relativně vysoká sorpce tebuconazolu na regozem byla pravděpodobně způsobena vyšším obsahem a jiným složením jílových částic a vyšším obsahem hydrofobní neutrální organické hmoty. Dalším vysvětlením je podobný obsah oxyhydroxidů Fe a Mn v obou půdách, z čehož vyplývá, že důležitými složkami půd ovlivňující sorpci tebuconazolu, je také zastoupení těchto půdních komponent. Podobně jako při experimentu s vybranými minerály i v případě kontrastních typů půd byla s výjimkou kambizemě vyšší sorpce zaznamenána, pokud byl tebuconazole použit ve formě komerčně dostupného prostředku. K podobným závěrům ve svých studiích dospěli i Pose-Juan et al. (2010) a Rodríguez-Cruz et al. (2006).

5.2.3 Sorpce tebuconazolu ve formě komerčně dostupného přípravku na kontrastní typy půd, humínové kyseliny a synteticky připravený minerál ferrihydrit v přítomnosti Cu

Posledním cílem bylo objasnit, zda přítomnost Cu či tvorba Cu-azol komplexů, mohou ovlivňovat sorpci tebuconazolu (ve formě komerčně dostupného přípravku) na půdní částice či minerály. Sorpce tebuconazolu na kambizem, regozem a humínové kyseliny při přídavku jednotlivých koncentrací Cu byla následující: poměr Teb : Cu 1 : 4 > 1 : 1 > 4 : 1 > vzorky bez přídavku Cu. Nejvyšší přídavek Cu opět vedl k nejvyšší sorpci tebuconazolu na dané matrice v důsledku tvorby Teb-Cu komplexů, které mají vyšší afinitu k jednotlivým půdním složkám (Arias et al., 2006). Důležitou roli opět hrála aditiva přítomná v komerčním produktu, která podpořila sorpci tebuconazolu na matrice obsahující organickou hmotu, a proto pravděpodobně nedocházelo ke kompetici tebuconazolu s Cu jako v případě standardní látky. Sorpce tebuconazolu na rendzinu a ferrihydrit měla následující trend: vzorky bez přídavku Cu > 1 : 4 > 1 : 1 ≈ 4 : 1. Sorpce tebuconazolu na ferrihydrit byla výrazně vyšší u vzorků, které neobsahovaly přídavek Cu. U vzorků, které obsahovaly přídavek Cu pravděpodobně docházelo ke kompetici organických aditiv s Cu o adsorpční místa. V přítomnosti Cu byla aditiva na ferrihydrit sorbována méně, s čímž následně souvisí i menší sorpce tebuconazolu na tento minerál. Na druhou stranu, u vzorků, které obsahovaly Cu v různých koncentracích, byl trend sorpce tebuconazolu na ferrihydrit: 1 : 4 > 1 : 1 > 4 : 1. Tento trend sorpce tebuconazolu na ferrihydrit byl pozorován i u rendziny. Důvodem může být právě vysoký obsah amorfních a slabě krystalických oxidů Fe v rendzině.

Sorpce tebuconazolu ve formě komerčně dostupného přípravku byla opět významně vyšší než v experimentech s tebuconazolem ve formě standardní látky. Jak již bylo zmíněno dříve, je to především způsobeno přítomností organických aditiv (např. N,N-dimethyldekanamid).

6. Závěry a doporučení pro využití poznatků v praxi

Z obdržených výsledků můžeme potvrdit, že mezi hlavní komponenty, které ovlivňují sorpci tebuconazolu patří obsah a kvalita organické hmoty. Nelze však opomenout také obsah a zastoupení minerálů a jílových částic, které ovlivňují sorpci tohoto fungicidu. Mezi další faktory, které ovlivňují sorpci tebuconazolu patří i hodnota pH, popř. přítomnost jiných prvků (např. Cu). Z důvodu schopnosti tebuconazolu tvořit komplexy s Cu, dochází pravděpodobně v přítomnosti Cu k jeho vyšší afinitě k půdním částicím a následně k vyšší sorpci tebuconazolu na půdu a popřípadě i k jeho následné akumulaci. Je taktéž nutno zdůraznit, že forma látky, ve které se daný fungicid vyskytuje, má zásadní vliv na sorpční mechanismus a následné chování dané látky v půdním profilu. Aditiva přítomná v komerčně dostupných prostředcích mohou zvyšovat afinitu účinné látky k půdním částicím a podporovat tak sorpci a akumulaci tebuconazolu v prostředí. Proto by s ohledem na všechny zmiňované faktory, měl být v zemědělské praxi brán zřetel na optimální výběr a aplikaci této látky vzhledem k daným charakteristikám půd, používání pesticidních látek v minulosti, ale i klimatickým a hydrogeologickým vlastnostem daného regionu.

V reálných podmínkách bývá půda často kvůli častým ošetřením zemědělských plodin znečištěna směsí kontaminantů organického i anorganického původu. Proto by bylo vhodné zaměřit se a monitorovat interakce tohoto fungicidu s dalšími kontaminanty, které se do půdy dostaly při předešlých aplikacích.

Komerčně dostupné přípravky s účinnou látkou tebuconazole často obsahují další fungicidní organické látky. Při aplikaci těchto směsí může docházet ke kompetici jednotlivých aktivních látek o sorpční místa v půdě a následně zcela jinému mechanismu sorpce tebuconazolu. Bylo by tedy vhodné sledovat sorpci tebuconazolu a dalších fungicidů, které jsou obsaženy v jednotlivých směsích komerčně dostupných přípravků.

Na základě předešlých studií, je tebuconazole považován za fungicid, který je převážně sorbován ve svrchních půdních horizontech a nevykazuje tak rizika spojená s kontaminací spodních vod. I přesto ale byly naměřeny jeho koncentrace v povrchových vodách, které překračovaly limity dané evropskou unií pro pitnou vodu. Tyto údaje lze využít při dalších studiích, které by sledovaly možnost využití půdních aditiv (organické hmoty, jílových složek) ke zvýšení retence tebuconazolu v půdách, a tedy ke snížení možnosti negativních vlivů na životní prostředí spojených s aplikací tohoto fungicidu. Vhodná aditiva, která by bylo možno v běžné zemědělské praxi použít, by neměla představovat vysoké ekonomické náklady pro uživatele. Bylo by tedy zajímavé využít organický odpad z dalších zemědělských sektorů (např. odpady z produkce a ze zpracování ovoce, zeleniny, obilovin, vinné révy), potravinářství (výroba vína či piva), popř.

biologicky rozložitelný komunální odpad či odpad z produkce bioplynu a sledovat vliv přídavků těchto látek do půdy na mechanismus sorpce tebuconazolu, popř. jeho interakce s dalšími organickými či anorganickými látkami.

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8. Přílohy-publikované výsledky

8.1 Příloha 1

Název: Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects

Autoři: Michael Komárek, Eva Čadková, Vladislav Chrastný, François Bordas, Jean-Claude Bollinger

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8.2 Příloha 2

Název: Complexation between the fungicide tebuconazole and copper(II) probed by electrospray ionization mass spectrometry

Autoři: Jana Jaklová Dytrtová, Michal Jakl, Detlef Schröder, Eva Čadková, Michael Komárek

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Autoři: Eva Čadková, Michael Komárek, Regina Kaliszová, Věra Koudelková, Jiří Dvořák, Aleš Vaněk

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Název: Tebuconazole sorption in contrasting soil types

Autoři: Eva Čadková, Michael Komárek, Regina Kaliszová, Aleš Vaněk, Miluše Balíková

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8.5 Příloha 5

Název: The influence of copper on tebuconazole sorption onto soils, humic substances and ferrihydrite

Autoři: Eva Čadková, Michael Komárek, Regina Kaliszová, Jiřina Száková, Aleš Vaněk, François Bordas, Jean-Claude Bollinger

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8.6 Příloha 6

Název: pKa constant determination of two triazole pesticides: tebuconazole and penconazole

Autoři: Eva Čadková, Michael Komárek, Jean Debord, Loïc Della Puppa, François Bordas, Jean-Claude Bollinger

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Review article

Contamination of vineyard soils with fungicides: A review of environmental and toxicological aspects

Michael Komárek [a,b,*], Eva Čadková [a], Vladislav Chrastný [c,d], François Bordas [b], Jean-Claude Bollinger [b]

[a] Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 21, Prague 6, Czech Republic
[b] Groupement de Recherche Eau, Sol, Environnement (GRESE), Université de Limoges, 123, Av. Albert Thomas, 87060, Limoges, France
[c] Czech Geological Survey, Geologická 6, 152 00, Prague 5, Czech Republic
[d] Faculty of Science, University of South Bohemia, Branišovská 31, 370 05, České Budějovice, Czech Republic

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ABSTRACT

The contamination of agricultural soils with inorganic (Cu-based) and organic pesticides (including their residues) presents a major environmental and toxicological concern. This review summarizes available studies published on the contamination of vineyard soils throughout the world with Cu-based and synthetic organic fungicides. It focuses on the behavior of these contaminants in vineyard soils and the associated environmental and toxicological risks. The concentrations of Cu in soils exceed the legislative limits valid in the EU in the vast majority of the studied vineyards. Regarding the environmental and toxicological hazards associated with the extensive use of fungicides, the choice of fungicides should be performed carefully according to the physico-chemical properties of the soils and climatic and hydrogeological characteristics of the vine-growing regions.

Contents

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* Corresponding author. Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 21, Prague 6, Czech Republic. Tel.: +420 224 382 743; fax: +420 234 381 801.
E-mail address: komarek@af.czu.cz (M. Komárek).

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1. Introduction

Viticulture represents an important agricultural practice in many countries and the long-term use of organic and inorganic pesticides in vineyards has resulted into increased concentrations of these pollutants in soils and other environmental compartments (e.g., Flores-Vélez et al., 1996; Ribolzi et al., 2002; Hildebrandt et al., 2008). Contamination with metals and organic pollutants, together with erosion and tillage, reduces the quality of the soils and poses important environmental and toxicological threats. Vineyard soils are usually highly degraded soils in terms of biochemical properties (Miguéns et al., 2007) and are thus more susceptible to contamination. During the last few decades, some European vineyards have been abandoned, mostly those situated on steep slopes, which has led to intensive soil erosion and subsequent dispersion of the pollutants into the environment (Nóvoa-Muñoz et al., 2007a; Fernández-Calviño et al., 2008a). Although synthetic organic fungicides are banned in European organic viticulture, Cu-based fungicides, such as $Cu(OH)_2$, copper oxychloride $3Cu(OH)_2 \cdot CuCl_2$, $CuSO_4$ and Cu_2O, are allowed and indispensable for organic vine cultivation, at limited doses, however (8 kg Cu ha^{-1} which should be further decreased to 6 kg Cu ha^{-1} after four years of vine cultivation) (EC regulation 473/2002). Generally, the intensive use of fungicides in vineyards is currently a cause of public concern, because of the resulting presence of pesticide residues in water and wine products used for human consumption (Jacobson et al., 2005). The aim of this review is to summarize scientific literature published on the contamination of vineyard soils with inorganic and organic fungicides and to evaluate the associated environmental and toxicological hazards.

2. Copper-based fungicides in viticulture

Copper-based fungicides (such as the Bordeaux mixture, $CuSO_4 + Ca(OH)_2$) have been intensively used in Europe since the end of the 19th century to control vine (*Vitis vinifera* L.) fungal diseases, such as downy mildew caused by *Plasmopara viticola*. Additionally, other Cu compounds have been introduced including Cu-oxychloride $3Cu(OH)_2 \cdot CuCl_2$, $CuSO_4 \cdot 3Cu(OH)_2$, Cu_2O, $Cu(OH)_2$, etc., and their long-term application and subsequent wash-off from the treated plants have resulted into extensive Cu accumulation in vineyard soils. Besides vineyards, Cu-based fungicides have also been extensively used, e.g., in hop fields (Schramel et al., 2000; Komárek et al., 2009a), coffee (Loland and Singh, 2004), apple (Li et al., 2005), avocado orchards (Van Zwieten et al., 2004) and during the cultivation of several vegetables (e.g., tomatoes, potatoes) (Adriano, 2001).

2.1. Behavior of Cu in vineyard soils

Background Cu concentrations in unpolluted soils are influenced by the parent material from which the soils are formed and reach an average of 30 mg kg^{-1} (Adriano, 2001; Baize, 1997). Its solubility, as of other metals, is greatly dependent on soil pH and will be most readily available at pH values below 6 (Adriano, 2001; Celardin et al., 2004; Boudesocque et al., 2007). In acidic vineyard soils (e.g., developed on granitic rocks), Cu can migrate throughout soil profiles more easily and thus cause groundwater pollution (Nóvoa-Muñoz et al., 2007a). On the other hand, Cu mobility in soils can increase at pH values above ~7.5 due to the solubilization of soil organic matter (SOM) and formation of Cu-SOM complexes (Arias et al., 2006; Karlsson et al., 2006; Fernández-Calviño et al., 2008b, 2009a). Copper in soils is mostly associated with SOM, Fe-, Mn-(hydr)oxides and to a lesser extent with clay minerals through specific and non-specific adsorption (Parat et al., 2002; Bradl, 2004; Fernández-Calviño et al., 2009b). Such strong sorption/complexation properties make it one of the least mobile metals in soils. However, metals of anthropogenic origin present in general a greater mobility in soil comparatively to a natural origin where the metals are strongly associated with soil components (Baize, 1997). When Cu enters the soil, as a result of the wash-off from vine leaves (Paradelo et al., 2008) and accidental spills of the fungicides, its speciation rapidly changes and Cu is sorbed and (co)precipitated in the soil. This redistribution of Cu into less available chemical fractions of soils which decreases its mobility and bioavailability is referred to as "aging" (Ma et al., 2006; Arias-Estévez et al., 2007a; Sayen et al., 2009).

The affinity of Cu to separate soil fractions decreases in the following order: Mn-(hydr)oxides > SOM > Fe-(hydr)oxides > clay minerals (Bradl, 2004). Similar sequences, SOM > Fe-, Al-oxyhydroxides > clays and SOM > silicate clays > ferrihydrite, were observed by McLaren and Crawford (1973) and Martínez-Villegas and Martínez (2008), respectively. However, in soils with a significantly higher content of Fe-(hydr)oxides (especially ferrihydrite), these can present the most important sink for Cu (Bradl, 2004). Sorption on SOM by means of complexation especially with humic and fulvic acids presents possibly the most important retention mechanism for Cu in soils (if not the most important one) (Strawn and Baker, 2008, 2009). Its association with SOM through inner-sphere complexation (e.g., bidentate inner-sphere coordination with carboxyl or amine ligands; Strawn and Baker, 2008) results in its lower toxicity compared to free Cu^{2+} (Karlsson et al., 2006). Additionally, Cu-rich SOM is less vulnerable to biodegradation (Parat et al., 2002). In most cases, the sorption of Cu in soils follows well either the Langmuir or the Freundlich isotherm (Arias et al., 2004; Bradl, 2004; Komárek et al., 2009b).

However, SOM can influence the mobility of Cu by two different means: while particulate SOM will act as a sorbent for Cu, soluble SOM will actually complex Cu, increasing thus its solubility, especially at alkaline pH (above ~7.5) (Arias et al., 2006; Fernández-Calviño et al., 2008a; Martínez-Villegas and Martínez, 2008). Besnard et al. (2001) found that clay minerals and organo-clay associations present in the <2 mm fractions, as well as particulate organic matter (POM) were the main carrier phases of Cu in soils. Flores-Vélez et al. (1996) found that Cu was mainly associated with the coarse light organic fraction and fine clay fraction in an acid sandy vineyard soil. The finer fractions, and particularly organic or inorganic colloids, play a major role in the accumulation and the mobilization of potential toxic elements such as metals in soils and waters (Ajmone-Marsan et al., 2008; Pédrot et al., 2008). For example, the formation of soluble organic complexes reduces the lability of Cu but under field conditions, it could enhance its mobility through the soil (Martínez and McBride, 1999; Martínez et al., 2003).

Soil carbonates proved to be another important factor controlling Cu mobility (and thus bioavailability) in soils. Ponizovsky et al. (2007) found that the activity of Cu in calcareous soils is to a great extent controlled by the surface precipitation of $CuCO_3$. This is especially important in alkaline soils containing high concentrations of carbonates, which is the case for many vineyards. The retention of Cu in calcareous soils through co-precipitation with carbonates is associated with the release of Ca^{2+}, Mg^{2+}, Na^{+} and H^{+} into the soil solution at equimolar ratios (Ponizovsky et al., 2007). The precipitation of newly-formed Cu phases in the soil presents thus an important retention mechanism of Cu retention in soils. These phases include $Cu(OH)_2$, $CuCO_3/Cu_2(OH)_2CO_3$, CuO and in the presence of sulfates (e.g., from the Bordeaux mixture), precipitation of various Cu-hydroxysulfates (Ma et al., 2006; Ponizovsky et al., 2007; Komárek et al., 2009b; Strawn and Baker, 2009). The solubility of Cu minerals in soils decreases in the following order $CuCO_3 > Cu_3(OH)_2(CO_3)_2$ (azurite) $> Cu(OH)_2 > Cu_2(OH)_2CO_3$ (malachite) > CuO (tenorite) > $CuFe_2O_4$ (cupric ferrite). Copper sulfates, such as $CuSO_4$ (chalcocyanite) and $CuSO_4 \cdot 5H_2O$ (chalcanthite), are highly soluble and require very high Cu concentrations to form in soils (Lindsay, 1979). Phosphate application to soils (e.g., as fertilizers) can reduce the mobility of risk metals through sorption mechanisms on newly formed phases, but probably not through precipitation of Cu-phosphates as observed, for example, for Pb-phosphates (Cao et al., 2003; Pérez-Novo et al., 2009).

Methodologies involving the use of different chemical reagents (e.g., one-step extractions, sequential extractions) do not provide information about the actual binding form, but rather differentiate metals into separate operationally-defined fractions (e.g., exchangeable, reducible, oxidizable, residual etc.) (Gleyzes et al., 2002). In other words, these protocols allow defining pools of extractable metals in particular chemical conditions. Sequential extractions are only a tool for predicting the chemical fractionation of Cu in soils and the results obtained are not sufficient for determining its actual geochemical position in soils, due to the lack of selectivity of the extractions and because several reactions are taking place during the procedure (complex formation, redistribution and precipitation reactions) (Nirel and Morel, 1990; Flores-Vélez et al., 1996). Nevertheless, it is needed to point out that sequential and one-step extractions are time-efficient methods that allow, when carefully interpreted, comparison of results from different works.

As in other contaminated soils, Cu in vineyard soils is mainly associated with the oxidizable and, to a lesser extent, with the reducible soil fraction, according to the SOM and oxide contents in the soils (e.g., Arias et al., 2004; Nóvoa-Muñoz et al., 2007a; Fernández-Calviño et al., 2008b; Komárek et al., 2008). The results of the sequential extraction could be completed by additional data about the binding forms, speciation and mineralogy, obtained through advanced spectroscopic and microscopic methods: X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), X-ray absorption near edge structure (XANES), extended X-ray absorption fine structure (EXAFS), X-ray fluorescence (XRF) and scanning microscope (SEM) analyses (Flores-Vélez et al., 1996; Boudesocque et al., 2007; Sipos et al., 2008; Strawn and Baker, 2008; Sayen et al., 2009).

2.2. (Bio)availability and toxicity of Cu in vineyard soils

The phytotoxicity of Cu is the highest in acidic soils with a low cation exchange capacity. Chaignon et al. (2003) observed significantly higher Cu concentrations in tomato (*Lycopersicon esculentum* Mill.) roots grown on strongly acidic soils compared to calcareous or mildly acidic soils with the same Cu concentration. Nevertheless, Cu phytotoxicity to agricultural plants grown on calcareous soils from former vineyards has been observed as well (Michaud et al., 2007). Although Cu concentrations in roots are a good indicator of Cu bioavailability in soils (Brun et al., 2001; Chopin et al., 2008), this time-consuming approach is not suitable for routine analyses. Furthermore, it is needed to point out that Cu uptake by roots is species-dependent and influenced by root type and size (i.e., fine vs. coarse roots) (Brun et al., 1998; Chopin et al., 2008). Total Cu concentrations alone do not provide adequate information about the bioavailability of the metal in soils as well (Wightwick et al., 2008). Water-soluble Cu concentrations alone do not give sufficient information either, because a portion of exchangeable Cu (not extractable by water) in soils can be easily taken up by roots. Plants can influence the (bio)availability of metals in their rhizosphere, for example Dessureault-Rompré et al. (2008) showed that root exudation could lead to the mobilization of SOM which complexes and solubilizes Cu. In other cases, plants are able to transform Cu into metallic nanoparticles in and near roots to prevent Cu toxicity (Manceau et al., 2008).

For a fast and simple prediction of Cu (bio)availability, common soil testing procedures, such as extractions using 0.1 M $CaCl_2$, 0.05 M EDTA (ethylenediaminetetraacetate) and 0.005 M DTPA (diethylenetriaminepentaacetate), are usually sufficient (Brun et al., 1998; 2001; Chaignon et al., 2003; Michaud et al., 2007; Komárek et al., 2008), despite the fact that the EDTA extraction often overestimates bioavailable Cu due to the dissolution of various soil phases (Arias et al., 2004; Nóvoa-Muñoz et al., 2007a; Komárek et al., 2008). It remains, however, a fast and sufficiently accurate method for determining potentially available risk metals in soils (Chrastný et al., 2008).

The extracting solution used for predicting the potential (bio) availability of Cu in soils should thus be chosen carefully according to specific soil characteristics and the results must be interpreted in accordance with the extractant used. For example, Brun et al. (1998) recommended the 0.1 M $CaCl_2$ extraction as the best suitable for the determination of Cu (bio)availability in acid-neutral vineyard soils due to a good correlation with Cu contents in wild growing plants. On the other hand, this extraction is not suitable for alkaline soils and total soil Cu combined with other extraction procedures, such as 0.05 M EDTA or 0.005 M DTPA are preferable as indicators of Cu (bio) availability (Brun et al., 2001; Chaignon et al., 2003; Komárek et al., 2008). The use of Diffusion Gradient in Thin-films (DGT) could be a supplementary tool to assess metal (bio)availability in soils and quantify the labile pool (Zhang et al., 1998; Nowack et al., 2004; Ruello et al., 2008). However, differences could appear between the result of the DGT and the concentrations in the soil solution because of the presence of colloidal fractions (Ruello et al., 2008).

The toxicity of Cu is influenced by its chemical species, while Cu^{2+} is the most toxic species, $Cu(OH)^+/Cu(OH)_2$ is significantly less toxic, Cu-carbonates ($CuCO_3$, $CuHCO_3^+$, $Cu(CO_3)_2^{2-}$) and chloro-complexes are almost non-toxic at all (Devez et al., 2005). As mentioned before, the complexation of Cu with SOM reduces significantly its toxicity as well (Karlsson et al., 2006). Karlsson et al. (2006) showed that in an organic soil, less than 0.2% of total Cu was in the free form (Cu^{2+}) at pH 4.8–6.3. On the other hand, there has been evidence of labile lipophilic organic Cu complexes which were able to pass through the cell membrane resulting thus into toxic effects. Such complexes can be formed with organic compounds (e.g., pesticides) that can be present in vineyard soils (Florence et al., 1992; Devez et al., 2005).

Increased metal concentrations in soils negatively influence the sustainability of agroecosystems. Earthworm populations are sensitive to tillage and Cu/Zn pesticides residues (Paoletti et al., 1998; Eijsackers et al., 2005); therefore, they present useful reliable bioindicators of the stability of vineyard agroecosystems. Belotti (1998) set the critical Cu concentration in soils (above which populations of several earthworm species are negatively affected) to 33 mg kg^{-1}, a value easily met in most studied vineyards (Table 1). Helling et al. (2000) published even a lower concentration of Cu originating from Cu-oxychloride (16 mg kg^{-1}) which had a detrimental impact on populations of the earthworm *Eisenia fetida*. In another study, Snyman et al. (2009) observed measurable toxicological responses of the snail *Helix aspersa* to Cu-oxychloride treatments. However, the chemical form of the Cu compound used influences its toxicity. El-Gendy et al. (2009) showed that the oxidative stress caused by different Cu compounds in the snail *Theba pisana* decreased in the following order: Cu-sulfate > Cu-hydroxide > Cu-oxychloride. Furthermore, it seems that soil microorganisms (including fungi) are able to transform Cu-oxychloride into insoluble Cu-oxalate which significantly reduces the toxicity of Cu in soils (Gharieb et al., 2004). Different toxicological tests (on *Vibrio fischeri*, *Pseudokirchneriella subcapitata* and *Daphnia magna*) showed that common environmental concentrations of Cu-based fungicides can pose a risk for other non-

Table 1
Copper concentration in upper layers of vineyard soils as published in literature.

Country	Depth (cm)	mg Cu kg^{-1}	Method used	Reference
Australia	0–1	9–249	15.5 M HNO_3	Pietrzak and McPhail (2004)
Australia	0–10	6–223	HNO_3+HCl	Wightwick et al. (2008)
Brazil	0–20	51–665	$H_2O_2+HClO_4+HF$	Casali et al. (2008)
Brazil	0–5	37–3216	HNO_3+HClO_4+HF	Mirlean et al. (2007)
Brazil	0–5	433–517	HNO_3+HClO_4+HF	Mirlean et al. (2009)
Bulgaria	0–10	72	HNO_3+HCl	Angelova et al. (1999)
Canada	0–15	10–77	HNO_3	Frank et al. (1976)
Croatia	0–20	105–553	$HClO_4+HNO_3+HCl+HF$	Miko et al. (2007)
Croatia	0–10	30–700	HNO_3+HCl	Romić et al. (2004)
Czech Republic	0–20	20–168	$O_2+O_3+NO_x$ at 400 °C + HNO_3+HF	Komárek et al. (2008)
France	0–10	248–378	$HClO_4+HF$	Besnard et al. (2001)
France	0–15	14–251	HNO_3+HCl	Brun et al. (1998, 2001)
France	0–15	158	HNO_3+HCl	Brun et al. (2003)
France	2–15	144	$HClO_4+HF$	Chaignon et al. (2002)
France	0–30	22–398	$HClO_4+HF$	Chaignon et al. (2003)
France	5–10	232	$HNO_3+HCl+HF$	Chopin et al. (2008)
France	0–10	17–34[a]	$HClO_4+HF$	Dousset et al. (2007)
France	0–2	323	HF	Flores-Vélez et al. (1996)
France	0–10	15–430	$HClO_4+HF$	Jacobson et al. (2005)
France	n.a.	32–184 (1030)[b]	$HClO_4+HF$	Michaud et al. (2007)
France	0–20	57–332	$LiBO_2$ at 550 °C + HNO_3	Parat et al. (2002)
France	0–10	89–243	HNO_3	Probst et al. (2008)
France	0–25	55–115	$HClO_4+HCl+HF$	Ribolzi et al. (2002)
Georgia	AB horizons	137–398	HNO_3+HCl	Narimanidze and Brückner (1999)
Greece	0–30	<157	HNO_3+HCl	Vavroulidou et al. (2005)
Italy	0–15	50–300[c]	HNO_3+HClO_4	Bretzel and Calderisi (2006)
Italy	0–30	183	n.a.	Cattani et al. (2006)
Italy	n.a.	215–372	HNO_3+HCl	Dell'Amico et al. (2008)
Italy	0–10	<945	HNO_3+HCl	Deluisa et al. (1996)
Italy	n.a.	125–155[c]	HNO_3+HClO_4	Paoletti et al. (1998)
Moldova	Surface	<250	n.a.	Perel'man et al. (1980)
New Zealand	0–10	4–259 (304)[d]	HNO_3+HCl	Morgan and Taylor (2004)
Portugal	0–30	58–130	n.a.	Magalhães et al. (1985)
Slovenia	0–20	163	n.a.	Kos and Leštan (2004)
Slovenia	0–45	364	n.a.	Pociecha and Lestan (2009)
Slovenia	0–20	65–99	HNO_3+HCl	Rusjan et al. (2006)
Slovenia	0–20	87–120	HNO_3+HCl	Rusjan et al. (2007)
Spain	0–20	40–301	$HNO_3+HCl+HF$	Arias et al. (2004)
Spain	0–20	179–549	$HNO_3+HCl+HF$	Arias et al. (2005a)
Spain	0–20	60–560	$HNO_3+HCl+HF$	Arias et al. (2006)
Spain	0–20	35–550	$HNO_3+HCl+HF$	Díaz-Raviña et al. (2007)
Spain	0–5	42–583	$HNO_3+HCl+HF$	Fernández-Calviño et al. (2008a)
Spain	0–20	79–130	$HNO_3+HCl+HF$	Fernández-Calviño et al. (2008b)
Spain	0–20	25–272	$HNO_3+HCl+HF$	Fernández-Calviño et al. (2008c)
Spain	0–20	61–434	$HNO_3+HCl+HF$	Fernández-Calviño et al. (2008d)
Spain	0–20	55–112	$HNO_3+HCl+HF$	Fernández-Calviño et al. (2009a,b)
Spain	0–10	125–603	$HNO_3+HCl+HF$	Nóvoa-Muñoz et al. (2007)
Spain	0–5	42–583	$HNO_3+HCl+HF$	Pateiro-Moure et al. (2007)
Spain	0–20	38–63	HNO_3+HCl	Ramos (2006)
Switzerland	n.a.	58–489	2 M HNO_3	Celardin et al. (2004)
Thailand	0–10	115–238	X-ray fluorescence	Joannon et al. (2001)

[a] Newly-planted vineyards.
[b] Localized contamination with occasional spills of fungicides.
[c] Approximate values estimated from figures.
[d] Vineyard in former orchard.

target organisms; however, when combinations of pesticides were tested, Cu showed antagonistic action towards synthetic organic pesticides (including metalaxyl), which resulted into lower toxicities than expected (Kungolos et al., 2009).

Increased Cu concentrations affect adversely, in terms of numbers and variability, the microbial communities present in vineyard soils (Dumestre et al., 1999; Díaz-Raviña et al., 2007; Dell'Amico et al., 2008; Lejon et al., 2008). Not only natural processes taking place in soils are negatively influenced, but the reduction of microorganism activity and/or changes in microbial populations can lead to lower mineralization rates of organic xenobiotics, such as organic pesticides. It is needed to point out that the contamination of vineyard soils with Cu is rather heterogeneous, creating thus “hotspots” of increased Cu concentrations within the soil (e.g., at places with increased SOM contents). There has been evidence that microorganisms are able to avoid these “hotspots” or that metal-tolerant species colonize them preferably (Yamamoto et al., 1985; Jacobson et al., 2007).

Significantly higher Cu concentrations have been observed in roots compared to shoots of several plants grown on Cu-enriched soils (Chaignon et al., 2003; Cattani et al., 2006). This can be explained by the fact that plants can, to some extent, actively control the uptake (e.g., lower pH of the rhizosphere compared to bulk soil, root exudates etc.) and translocation of Cu as a micronutrient. Furthermore, Cu is mainly fixed in the apoplasm due to its affinity for the negatively charged components of root cell walls and is not taken up into the root cells (Crawley, 1997). Copper uptake by plants is also dependent on phytosiderophores released by plant roots. Chaignon et al. (2002) observed that under Fe and Zn deficiency, wheat plants (*Triticum aestivum* L.) acquired elevated concentrations of Cu probably due to the increased release of phytosiderophores. Increased concentrations of available Cu in soils has been shown to cause rhizotoxic effects to durum wheat (*Triticum turgidum durum* L.) associated with reduction of root length and a significant decrease of Fe uptake which led to chlorosis symptoms (Michaud et al., 2008).

Only in rare cases, vines suffer from high Cu concentrations in vineyard soils mainly due to the fact that vine plants are deeply rooting and the highest concentrations of Cu are present in the superficial soil horizons. Elevated concentrations of potentially toxic metals in vines have been thus mostly attributed to atmospheric deposition (Angelova et al., 1999; Mihaljevič et al., 2006). Low Cu accumulation ratios by vine roots have been also documented by Chopin et al. (2008), with finer roots exhibiting higher Cu accumulation compared to coarse ones. Nevertheless, Brun et al. (1998; 2003) found that increased Cu concentrations affected the phenology, growth and reproduction of some ruderal plant species grown at vineyards, so it is possible to assume that plants with shallow roots and young vines are more susceptible to increased Cu concentrations. Therefore, changing the agricultural practices, such as replacing the vines with young ones, with other shallow rooting crops or pasture plants, could lead to phytotoxic effects and contamination of the newly grown plants.

2.3. Contamination of vineyard soils with Cu

Due to the fact that Cu is strongly immobilized by SOM and Fe-, Mn-(hydr)oxides, Cu concentrations in superficial horizons of vineyard soils often exceed 200 mg Cu kg^{-1} (e.g., Flores-Vélez et al., 1996; Brun et al., 1998, 2001; Narimanidze and Brückner, 1999; Pietrzak and McPhail, 2004; Table 1). The highest Cu concentrations are present in the upper layers of soil profiles and the closest to the vine plants (Magalhães et al., 1985; Pietrzak and McPhail, 2004; Fernández-Calviño et al., 2008c; Wightwick et al., 2006; Komárek et al., 2008). For example, Arias et al. (2004) found that in Galicia, NW Spain, Cu concentrations exceeded 100 mg kg^{-1} in 60% of subsurface soil samples due to the application of Cu-based fungicides at vineyards. Higher Cu concentrations have been observed, as expected, in soils from older and especially abandoned vineyards compared to younger ones (Rusjan et al., 2006; Wightwick et al., 2006; Fernández-Calviño et al., 2008d; Komárek et al., 2008). Rusjan et al. (2007) further found that the highest Cu concentrations in Slovenian vineyard soils were present in vineyards located on terraces, followed by plateaus and planes. On the other hand, Magalhães et al. (1985) observed higher Cu concentrations in vineyard soils located on planes compared to terraces. Table 1 and Fig. 1 summarize data obtained from the literature on Cu concentrations in upper layers of vineyard soils throughout the world. Most of the values published exceed the warning and critical legislative limits valid in the EU which set Cu concentrations in agricultural soils at 50 and 140 mg kg^{-1}, respectively (values above which the application of sewage sludge is not suitable; Council Directive 86/278/EEC, 1986).

The low mobility of Cu in soils has been generally accepted (except extreme acidic conditions such as in podzols, Baize, 1997). However, Pietrzak and McPhail (2004) found >50% of fungicide-derived Cu present in the potentially available fraction (water extractable + exchangeable + adsorbed fraction) of subsurface vineyard soil horizons. The conversion to less mobile phases ("aging") can be slow which can present environmental concerns. In France, the intensive use of the Bordeaux mixture led in some cases to the accumulation of Cu concentrations above 1000 mg kg^{-1} (Flores-Vélez et al., 1996). Extremely high concentrations (>3000 mg Cu kg^{-1}) in Brazilian vineyard soils (100-year old vineyard) have been documented by Mirlean et al. (2007). These values are, so far, the highest published and indicate that the intensive use of pesticides in areas of wet subtropical climate results into significantly higher Cu concentrations in soils compared to drier areas. Statistical treatment of data (one-way ANOVA, Duncan test) concerning maximum Cu concentrations in different countries showed that there is a significant difference ($p < 0.05$) between Brazilian and European vineyards, with the only exception of Spain where Cu concentrations were statistically similar to both regions. Such increased Cu concentrations pose a significant risk for groundwater (Mirlean et al., 2009). The influence of climate is evident as higher pesticide doses have to be applied in more humid regions due to the more intense development of downy mildew. For example, vineyard soils in the drier Mediterranean region of France contained significantly lower concentrations than those in the more humid region of Champagne (France) (Brun et al., 1998; Besnard et al., 2001). The same evolution was observed in Italy, where significantly higher Cu concentrations were observed in the humid mountainous regions compared to dry areas in Southern Italy (Deluisa et al., 1996). Maximum Cu concentrations in vineyard soils from different regions related to rainfall are presented in Fig. 1.

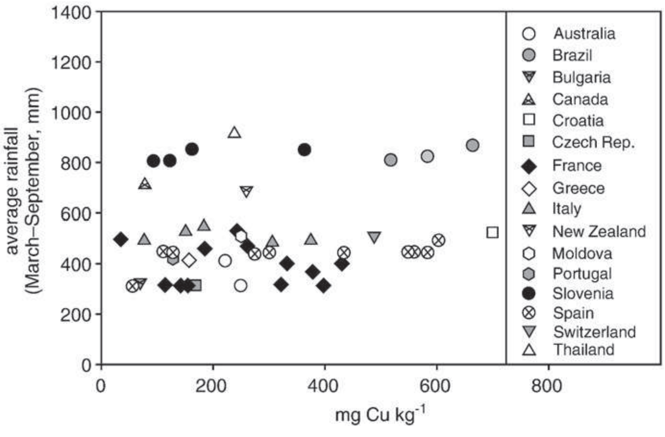

Fig. 1. Maximum Cu concentrations in vineyard soils originating from different regions related to average rainfall in March–September (vine-growing period) as obtained from literature and WMO (World Meteorological Organization; http://www.worldweather.org). Outlying data were excluded.

It has been estimated that a single application of the Bordeaux mixture in the region of Champagne (France) introduced 3–5 kg Cu ha^{-1} with 3–10 applications per year, which resulted into average Cu concentrations ranging from 100 to 1500 mg kg^{-1}. However, only ~60% of Cu was retained in the soils, suggesting that losses must have occurred (Brun et al., 1998; Besnard et al., 2001). Because many vineyards are located on steep slopes, intensive erosion further influences Cu mobility in these soils and thus increases the risks associated with groundwater contamination (Ribolzi et al., 2002; Xue et al., 2003; Fernández-Calviño et al., 2008a). Copper applied to eroded vineyard soils can easily reach ground and surface waters either as water-soluble species or associated to colloidal soil particles and concentrate in surface water sediments (Fernández-Calviño et al., 2008b). Furthermore, when Cu enters surface waters, it can be toxic to aquatic organisms (Zyadah and Abdel-Baky, 2000).

Among many, planting vegetation between vine rows is suggested as an effective anti-erosion precaution (Marques et al., in press). Organic soil amendments are often applied to increase soil fertility and decrease soil erosion by runoff. The most commonly used amendments are bark, vine shoots and compost. These amendments can, however, influence Cu retention in soils through the introduction of POM to soils. Due to its chemical acid/base and complexing properties, POM plays an important role in both soil buffering and Cu sorption. Although a POM size effect was observed on the number of reactive surface sites, nothing such can be evidenced for Cu affinities toward POM fractions (Sébastia et al., 2008). Copper may be adsorbed (immobilized) on the surfaces of POM or complexed (mobilized) with its reactive functional groups (Flores-Vélez et al., 1996; Besnard et al., 2001; Devez et al., 2005). Nevertheless, the addition of organic matter to contaminated vineyard soils decreases the toxicity of Cu to microorganisms (Lejon et al., 2008). The main factors influencing the mobility of Cu in vineyard soils and the potential risk of ground and surface water pollution thus include: (i) soil pH, (ii) erosion and (iii) tillage (Arias et al., 2004; 2006; Besnard et al., 2001; Mirlean et al., 2007; Nóvoa-Muñoz et al., 2007a; Fernández-Calviño et al., 2008a).

In addition to fungicides, Robinson et al. (2006) revealed that vineyard posts treated with Cu–Cr–As can be another source of Cu in vineyard soils. Nevertheless, the study by Ko et al. (2007) did not confirm that this treatment would lead to a contamination of vine plants. The application of wastes from wineries, such as bentonite and waste perlite improves soil properties (e.g., soil retention capacity, nutrient content, pH), but on the other hand can also increase risk metal (especially Cu) concentrations in soils (Arias-Estévez et al., 2007b; Nóvoa-Muñoz et al., 2008). Apart that, there has been evidence that the addition of compost can increase Cu (together with other metals) concentrations in vineyard soils as well (Ramos, 2006). Besides Cu, increased concentrations of other risk elements were found in vineyard soils. These include Zn (e.g., from fungicides, manure and compost applications; Weingerl and Kerin, 2000; Ramos, 2006), As (e.g., from herbicides, insecticides or geological background, Villaescusa and Bollinger, 2008; Nóvoa-Muñoz et al., 2007b), Pb (from atmospheric depositions, Mihaljevič et al., 2006; Komárek et al., 2008; but also from the use of lead arsenate as insecticide; Frank et al., 1976) and Cd (from phosphate fertilizers; Komárek et al., 2008).

2.4. Impact of Cu on health

Copper is mainly absorbed through the gastrointestinal tract and partially excreted within faeces. Another part is transported to the liver. An assessment of health effects to humans indicates that Cu is both an essential and a toxic element (WHO, 1998; Pichard et al., 2005). Copper in drinking water comes mainly from private Cu pipes connected to public water supplies, but water from wells located near vineyards can possibly contain increased Cu concentrations. The European directive 98/83/CE (EC, 1998a), relative to drinking water, sets Cu concentrations below 2.0 mg L^{-1}. Although epidemiological studies indicate the absence of negative health effects to children from chronic exposure at 0.8 mg Cu L^{-1} or more (maximum value: 4.2 mg Cu L^{-1}) (Zietz et al., 2003), acute exposure of adult volunteers (more than 10 mg Cu L^{-1}) led to rapid gastric diseases (Araya et al., 2003). Copper homeostasis by liver and its binding to some dedicated peptides and proteins are the key mechanisms to its bio-inorganic chemistry (Brown and Kozlowski, 2004). It is currently well admitted that these mechanisms play a critical role for the development of a series of neurodegenerative diseases like Alzheimer's, Parkinson's, Creutzfeldt–Jakob's ("Mad cow") diseases or Amyotrophic Lateral Sclerosis (Gaggelli et al., 2006).

The exposition to different fungicides presents a toxicological risk for workers in agriculture (Remor et al., 2009). There have been evidences that vineyard workers may develop serious acute and chronic respiratory problems, including lung carcinoma, due to the inhalation of Cu-containing fungicides (especially the Bordeaux mixture) (Pimentel and Marques, 1969; Zuskin et al., 1997; Santić et al., 2005). Besides Cu, other compounds containing risk elements used in viticulture can create toxicological risks. The high exposure of vineyard workers to As (present in carcinogenic fungicides) led to the banning of As-containing chemicals at vineyards in several countries (e.g., France) (Grillet et al., 2004).

3. Synthetic organic fungicides in viticulture

Synthetic organic fungicides used worldwide for vine protection come from different chemical classes and hence their adsorption and transport in the environment vary depending upon their physico-chemical properties (Wheeler, 2002). Their extensive use in viticulture has resulted into the contamination of surrounding environmental compartments, including surface and groundwaters. For example, Hildebrandt et al. (2008) reported the presence of fungicides in surface and groundwaters at the Ebro, Duero and Miño river basins devoted to vineyards and other agricultural practices at concentrations higher than the EU regulatory limit of 0.1 μg L^{-1} set for drinking water (EC, 1998a).

3.1. Behavior of synthetic organic fungicides in soils

The behavior, persistence and mobility of pesticides are closely associated with different processes occurring in soils: sorption–desorption, volatilization, chemical and biological degradation, uptake by plants and leaching (Arias-Estévez et al., 2008). Adsorption is one of the key processes that influence the concentrations of these pesticides in soil and soil solution and their movement through the soil profile (Kamrin, 1997; Gevao et al., 2000; Wheeler, 2002; Huang et al., 2003). In general, the sorption of pesticides correlates with their solubility in water (Tables 2 and 3), octanol–water partition coefficients (K_{ow}), distribution coefficients (K_d) and organic carbon partition coefficients (K_{oc}) (Kamrin, 1997). The adsorption of organic fungicides can be often described by the Freundlich and/or Langmuir isotherm (e.g., Fernandes et al., 2003; Arias et al., 2006; Pateiro-Moure et al., 2007). The K_{ow} coefficient basically indicates the distribution of a pesticide between water and octanol and gives an estimate of the partition between an aqueous and a lipophilic/organic phase. The higher K_{ow} is, the more likely a pesticide binds to organic matter in soils and sediments, or can enter living tissues. Values of log K_{ow} higher than 7 or 8 indicate strong adsorption and that these chemicals are almost immobile in the environment (Chamberlain et al., 1996; Baird, 2003). The log K_{ow} depends on pesticide ionisability and hence of its pK_a relative to the pH value of the soil solution (Chamberlain et al., 1996). The distribution coefficient K_d (L kg^{-1}) describes the distribution of the pesticide between the solid and the liquid phases, with higher K_d values indicating stronger adsorption to the soil matrix. While SOM is the main soil component involved in pesticide sorption, the K_{oc} is defined as the distribution coefficient for a particular soil and pesticide relative to the organic C content of the soil (Sukop and Cogger, 1992; Monkiedje and Spiteller, 2002a; López-Blanco et al., 2005). Generally, pesticides with higher K_{oc} values should present a lower risk for groundwater contamination; however, there has been also evidence of such contamination with pesticides with a K_{oc} value greater than 1000 (Arias-Estévez et al., 2008).

Table 2
Categories of fungicide solubility, persistence and mobility in soils (adapted from Kamrin, 1997; IUPAC, Pesticide Properties Database).

		Example
Solubility at 20–30 °C (mg L^{-1})		
Insoluble	<1	Quinoxyfen
Slightly soluble	1–100	Azoxystrobin, cyprodinil, fludioxonil, mancozeb, penconazole, procymidone, tebuconazole, vinclozolin
Soluble	100–10,000	Metalaxyl, propiconazole, pyrimethanil
Very soluble	>10,000 mg	
Pesticide half-life in soils (d)		
Low persistence	<30	Cyprodinil, mancozeb, metalaxyl, procymidone, propiconazole, pyrimethanil, quinoxyfen, vinclozolin
Moderate persistence	30–100	Azoxystrobin, cyprodinil, fludioxonil, metalaxyl, procymidone, propiconazole, pyrimethanil, quinoxyfen, tebuconazole
High persistence	>100	Fludioxonil, penconazole, pyrimethanil, quinoxyfen, tebuconazole
Pesticide mobility[a] *(K_{oc}; mL g^{-1})*		
Very mobile	<15	
Mobile	15–74	Metalaxyl
Moderately mobile	75–499	Metalaxyl, procymidone, pyrimethanil, vinclozoline
Slightly mobile	500–4000	Azoxystrobin, cyprodinil, mancozeb, penconazole, propiconazole, pyrimethanil, tebuconazole, vinclozoline
Non-mobile	>4000	Fludioxonil, quinoxyfen

[a] Mobility according to the SSLRC classification (Soil Survey and Land Research Centre, Cranfield University, UK).

Pesticides and their residues are adsorbed to soil particles, including SOM, Fe-, Mn-(hydr)oxides and various clay minerals. A lot of mechanisms are involved during the adsorption processes and all of them may be involved simultaneously. These include ionic, hydrogen and covalent bonding, charge-transfer or electron donor–acceptor mechanisms, van der Waals forces, ligand exchange, and

Table 3
Basic chemical characteristics of fungicides commonly used in viticulture (data from Tomlin, 2003; Kamrin, 1997; IUPAC, Pesticide Properties Database).

Fungicide	Chemical name	Chemical class	Water solubility (g L^{-1})	log K_{ow}	K_{oc} (L kg^{-1})	Soil half-life (d)
Azoxystrobin	methyl (*E*)-2-{2-[6-(2-cyanophenoxy)pyrimidin-4-yloxy]phenyl}-3-methoxyacrylate	Strobilurins	0.006 (at 20 °C)	2.5 (at 20 °C)	500	56
Cyprodinil	4-cyclopropyl-6-methyl-*N*-phenylpyrimidin-2-amine	Anilinopyrimidines	0.013 (at 25 °C)	4.0 (at 25 °C)	1706	20–60
Fludioxonil	4-(2,2,-difluoro-1,3-benzodioxol-4-yl)-1*h*-pyrrole-3-carbonitrile	Phenylpyrroles	0.0018 (at 25 °C)	4.1 (at 25 °C)	75,000	140–350
Mancozeb	zinc manganese ethylenebisdithiocarbamate	Dithiocarbamates	0.0062 (at 25 °C)	0.3	1000	1–7
Metalaxyl	methyl *N*-(methoxyacetyl)-*N*-(2,6-xylyl)-*DL*-alaninate	Phenylamides	8.4 (at 22 °C)	1.8 (at 25 °C)	30–300	10–40
Penconazole	(*RS*)-1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole	Triazoles	0.073 (at 25 °C)	3.7 (at 25 °C)	2205	133–343
Procymidone	*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane- 1,2-dicarboximide	Dicarboximides	0.0045 (at 25 °C)	3.1 (at 26 °C)	378	28–84
Propiconazole	(2*RS*,4*RS*;2*RS*,4*SR*)-1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-ylmethyl]-1*H*-1,2,4-triazole	Triazoles	0.1 (at 20 °C)	3.7 (at 25 °C)	950	29–70
Pyrimethanil	*N*-(4,6-dimethylpyrimidin-2-yl)aniline	Anilinopyrimidines	0.121 (at 25 °C)	2.8 (at 25 °C)	265–751	7–4
Quinoxyfen	5,7-dichloro-4-quinolyl 4 fluorophenyl ether	Quinolines	0.000116 (at 20 °C)	4.7 (at 20 °C)	15,415–75,900	11–454
Tebuconazole	(*RS*)-1-*p*-chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol	Triazoles	0.036 (at 20 °C)	3.7 (at 20 °C)	769	40–170
Vinclozolin	(*RS*)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione	Dicarboximides	0.0026 (at 20 °C)	3.0	100–735	3–21

hydrophobic bonding or partitioning (Gevao et al., 2000). Factors influencing pesticide adsorption involve physico-chemical properties of the compound (molecular size, molecular structure, ionisability, water solubility, lipophilicity and volatility) and soil properties (particle size, pH, SOM content and quality, oxyhydroxide content, clay content etc.) (Iglesias-Jiménez et al., 1997; Gevao et al., 2000; Clausen and Fabricius, 2001; Fernandes et al., 2006; Pateiro-Moure et al., 2009). Detailed sorption mechanisms and factors influencing pesticide sorption are summarized in the review by Gevao et al. (2000). The aging of organic (as well as inorganic) fungicides in soils may create stronger bonds between soil particles and the fungicides with increasing contact time which decreases pesticides availability for degradation and increases their half-life (Walker et al., 2005; Lerch et al., 2009; Sayen et al., 2009). Fungicide persistence in soils has been summarized by Kamrin (1997) (Table 2). More detailed information about the sorption, degradation and mobility of pesticides in soils is available in the review by Arias-Estévez et al. (2008). The following sections summarize the literature that has been published on the most intensively used synthetic organic fungicides in viticulture; however, not all the synthetic organic fungicides are mentioned due to the lack of literature sources.

3.1.1. Metalaxyl

Metalaxyl is an important fungicide with residual and systemic activity (Cohen et al., 1979). Soil half-life is estimated to be 10–40 d (Tomlin, 2003), although sunlight may increase metalaxyl degradation and decrease thus its half-life. Metalaxyl was found in soils (up to 1000 μg kg^{-1}) only shortly after its application which is probably caused by its high mobility in soils and relatively short life time (Bermúdez-Couso et al., 2007). Fernandes et al. (2003) have suggested that the adsorption of metalaxyl is affected mainly by SOM and soil colloids. In cases where the soil contains high contents of dissolved organic matter, adsorption may decrease or remain unaffected because of the competition between dissolved organic matter and the fungicide for adsorption sites (Fernandes et al., 2006). Other studies have shown different trends in metalaxyl adsorption. Sharma and Awasthi (1997) have reported that metalaxyl adsorption depends more on the clay content then on the SOM content. Sukop and Cogger (1992) state that SOM has little influence on metalaxyl adsorption as it is preferentially adsorbed on soil mineral surfaces. It was probably caused by the fact, that soils used in this study contained mostly sand and silt and were moderate in SOM. On the other hand, Andrades et al. (2001) have described the adsorption by both SOM and clays (with a predominant role of SOM). This assumption is supported by Monkiedje and Spiteller (2002a). More detailed information about metalaxyl degradation, adsorption, mobility and persistence are available in the review by Sukul and Spiteller (2000). Lowering the pH of the fungicide solution increases metalaxyl adsorption, the positively charged fungicide is then more attracted to the negative charge of soil components (Arias et al., 2006). A similar pattern was observed for penconazole and fludioxonil (Arias et al., 2005b).

The values of K_d for metalaxyl at 20 μM equilibrium concentration range from 0.64 to 1.76 L kg^{-1} with a maximum value of 9.17 L kg^{-1}. Lower values were obtained in soils containing moderate amount of SOM (1–1.3%) and clays (20–23%) and large amount of sand (54–71%) (Fernandes et al., 2003). When organic amendments were added to soils, K_d values increased in soils low in clays indicating the strong influence of SOM on fungicide sorption in soils (Fernandes et al., 2006). K_{oc} values in these soils ranged from 18 to 116 L kg^{-1}. The maximum value of 1135 L kg^{-1} was obtained in a soil rich in clay (68%), low in sand (0.3%) and with a low SOM content (0.8%) (Fernandes et al., 2006). Bermúdez-Couso et al. (2007) have shown a K_{oc} value of 50 L kg^{-1}. The changes in K_{oc} values depended on the origin and content of dissolved organic matter of the organic amendment (Fernandes et al., 2006). The values of K_d and K_{oc} indicate that the amount of SOM can significantly influence metalaxyl sorption, although it is needed to mention, that the origin, nature and type of SOM are probably the most important factors. Clay content also affects metalaxyl sorption. When soils contain large amounts of clay minerals compared to other soil components, the K_d and K_{oc} values increase (sorption increases). A similar pattern has been observed for cyprodinil, penconazole and propiconazole. This implies that soils used for viticulture rich in clay or SOM are preferable. Soils low in SOM should be fertilized with amendments rich in organic matter in order to avoid leaching of these fungicides to groundwater.

3.1.2. Fludioxonil, cyprodinil

Fludioxonil is a contact fungicide which is used against *Botrytis cinerea*. Cyprodinil (4-cyclopropyl-6-methyl-*N*-phenylpyrimidine) is a systemic fungicide used against rots. These pesticides are both considered as new generation fungicides and they are usually applied together (Arias et al., 2005b). Bermúdez-Couso et al. (2007) found concentrations of fludioxonil and cyprodinil in vineyard soils reaching 349 and 462 μg kg^{-1}, respectively. Concentrations of both fungicides in sediment samples from an adjacent river basin were much lower than those found in soils even though sediments rich in organic matter have a higher potential for fungicide sorption. Due to its lower aqueous solubility and mobility, lower fludioxonil concentrations were found in the sediments (compared to cyprodinil). The authors have assumed that the lower concentrations of fungicides found in sediments were caused by dilution, because sediments were collected in a basin that was devoted to areas with different agricultural use and hence with different fungicide use. Arias et al. (2005b) suggested that fludioxonil is adsorbed more intensively on soil particles than cyprodinil due to its higher K_d values (110–213 vs. 54–93 $\mathrm{L\,kg^{-1}}$). While the K_d value for fludioxonil did not depend on soil characteristics (SOM, clay content and pH), the K_d value for cyprodinil depended on the organic C content. K_{oc} values were also higher for fludioxonil (1671–5785 $\mathrm{L\,kg^{-1}}$) then cyprodinil (1679–2613 $\mathrm{L\,kg^{-1}}$), which is again in good agreement with the fact that fludioxonil is adsorbed in soils more intensively. Bermúdez-Couso et al. (2007) have stated that K_{oc} values for fludioxonil and cyprodinil varied from 991 to 2440 $\mathrm{L\,kg^{-1}}$ and from 1550 to 2030 $\mathrm{L\,kg^{-1}}$ in a sandy loam soil, respectively. The positive influence of SOM on the retention of these fungicides in soils suggests that the addition of organic matter or surfactants to soils could reduce the migration of hydrophobic pesticides (e.g., cyprodinil, penconazole) in soils and reduce thus the risks of ground and surface water contamination (Rodríguez-Cruz et al., 2006).

3.1.3. Penconazole

Penconazole is a systemic fungicide which is considered stable and tends to accumulate in soils (Singh, 2005). Bermúdez-Couso et al. (2007) found penconazole in soils (up to 411 $\mathrm{\mu g\,kg^{-1}}$) sampled throughout the year, with higher concentrations observed in summer and spring during the applications. Nevertheless, it is needed to point out that penconazole was found in the soils at relatively low concentrations which has been caused by its lower rate of application compared to the other fungicides. Singh (2005) has found that penconazole showed the highest adsorption intensity and the lowest mobility compared to the other triazole fungicides in soils with a low organic C content (0.4–0.5%). These results indicates that SOM content is not the only parameter affecting adsorption and hence other soil components (e.g., oxyhydroxides, clay minerals) can influence the adsorption of these fungicides in soils. The nature of the clays plays an important role as well (Singh, 2005). On the contrary, Rodríguez-Cruz et al. (2006) found that penconazole adsorbed to soils rich in SOM is more stable than penconazole adsorbed to natural soils with a low SOM content. Therefore, it possible to assume that SOM content is one of the most important factors influencing penconazole adsorption. Penconazole is much more intensely adsorbed to soils in the presence of Cu. This is probably due to the formation of $\mathrm{Cu^{2+}}$-penconazole complexes with a higher affinity for soil components (Arias et al., 2006).

3.1.4. Propiconazole

Propiconazole is a systemic fungicide used for protection against rust, powdery mildew and blight diseases. Its low mobility and relatively high adsorption in soils rich in organic matter result into its accumulation in soils and pose a risk for the soil ecosystem (Thorstensen et al., 2001). The majority of the applied propiconazole is retained in the top layers of soils, suggesting thus its low mobility. As in the case of penconazole, K_d values differ (0.96 and 2.84 $\mathrm{L\,kg^{-1}}$) in soils with a similar clay and silt content, suggesting again the influence of the clay minerals quality (Singh, 2005). Riise et al. (2001) have found that organic C was the most important factor in adsorption. The K_d values varied from 17.2 to 36.9 $\mathrm{L\,kg^{-1}}$; higher values were found for soil particles containing higher organic C content (1.6 vs. 0.4%). K_{oc} values ranged from 2244 to 5050 $\mathrm{L\,kg^{-1}}$. The highest value was observed in the finest fraction (<2 μm) although it contained the lowest amount of organic C (0.4%); therefore, the presence of other phases (e.g., Fe, Al oxides) and thus a higher specific surface area influences the adsorption. A similar pattern was found by Wu et al. (2003) where K_{oc} values in the colloidal fraction reached 8100 $\mathrm{L\,kg^{-1}}$. The K_d values found in their study varied from 27 to 113 $\mathrm{L\,kg^{-1}}$, where the colloidal fraction showed four time higher K_d values compared to the bulk sample. Kim et al. (2003) also studied the ability of propiconazole to accumulate in soils. The authors found that the half-life of propiconazole in a sandy loam soil with a low SOM content (0.8%) was 315 d, lower compared to >1 yr in a silty clay loam soil rich in SOM (3.2%). The formation of propiconazole bound residues contributes to the persistence of this fungicide in soil and is higher in soils containing higher SOM content (and further increases with time). On the contrary, propiconazole degradation and mineralization is less intensive in soils rich in SOM.

3.1.5. Vinclozolin, procymidone

Vinclozolin is strongly adsorbed on SOM even though its half-life in soils is estimated to be only 3–21 d (Kamrin, 1997). Successive applications of vinclozolin enhance the rate of its degradation (Slade et al., 1992). After the first application, the half-life was 22 d and the half-life decreased (rate of degradation increased) with successive applications to only 1 d after the third application. A similar pattern was also found for iprodione (Walker et al., 1986; Walker, 1987) and metalaxyl (Bailey and Coffey, 1985). The degradation intensity of vinclozolin increases with increasing soil pH (Walker, 1987; Ueoka et al., 1997). Therefore, the use of vinclozolin in alkaline soils, which is the case for many vineyards, does not have to pose a significant risk of leaching into the groundwater. According to the USEPA (2000), vinclozolin and its metabolites are very mobile–slightly mobile in sandy soils low in SOM. It is needed to mention that the use of vinclozolin is banned in some European countries such as Finland, Denmark and Norway (Milne, 2004).

Procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide] has a low mobility in soils rich in SOM (K_{oc} value 1500 $\mathrm{L\,kg^{-1}}$). However, the mobility increases during periods of increased precipitation. The concentration varies during the year as well, higher concentrations of procymidone were present in soils sampled in autumn and winter (up to 1124 $\mathrm{\mu g\,kg^{-1}}$) (Bermúdez-Couso et al., 2007). Even though procymidone is considered to be practically immobile, González-Pradas et al. (2002) have found procymidone occurrence in soils below 20 cm of the soil profile. The K_d values of procymidone ranges from 0.62 to 1.71 $\mathrm{L\,kg^{-1}}$ (González-Pradas et al., 2002). As for other fungicides, a higher clay content contributes to a higher K_d value and therefore to stronger adsorption. Despite the fact that procymidone is in the same chemical group as vinclozolin and iprodione, its successive applications did not have any effect on the rate of its degradation. This shows that procymidone is more stable in soils than the other two fungicides.

3.1.6. Azoxystrobin

Azoxystrobin belongs to a new class of widely-sold systemic fungicides called strobilurins, fungicides originating from natural products (Gullino et al., 2000; Bartlett et al., 2002). Azoxystrobin is stable in the pH range of 4–9 and is degraded only slowly by photolysis (FAO, 2007). Ghosh and Singh (2009) have shown that photodegradation plays an important role in its degradation. Azoxystrobin can persist in soils for several months and the degradation rate significantly correlates with soil pH and microorganism biomass (Bending et al., 2006, 2007). The K_{oc} values ranges from 207 $\mathrm{L\,kg^{-1}}$ in a sandy loam to 594 $\mathrm{L\,kg^{-1}}$ in a silty clay loam (EC, 1998b). According to the PMRA (Pest Management Regulatory Agency, 2007), K_{oc} values vary from 300 in loamy sand to 1690 in a silty clay loam. The average K_d value one day after azoxystrobin application to sandy loam soils containing 73% sand, 12% silt, 14% clay and 1.16–1.82% total organic C was 13.9 $\mathrm{L\,kg^{-1}}$ (Bending et al., 2007). The application of compost may decrease soil pH and increase organic C content, which leads to stronger sorption of azoxystrobin in the soil. Even though azoxystrobin is then less available to microbial degradation, the addition of compost together with anaerobic conditions enhances its degradation by anaerobic organisms (Ghosh and Singh, 2009) and azoxystrobin degradation products (e.g., (*E*)-2-(2-[6-cyanophenoxy)-pyrimidin-4-yloxyl]-phenyl-3-methoxyacrylic acid) can thus leach through the soil (FAO, 2007). Therefore, it was concluded that the use of azoxystrobin in areas where soils are permeable is not desirable as it may result in groundwater contamination (Bending et al., 2006). In such areas, azoxystrobin concentrations in surface waters can reach 30 μg L^{-1} (Berenzen et al., 2005).

3.1.7. Quinoxyfen

Quinoxyfen is an effective fungicide used on powdery mildews (Gullino et al., 2000). Quinoxyfen is believed to be persistent in the environment at alkaline pH and low temperature. It is immobile or only slightly mobile in soils depending on the soil type (EC, 2003a; CDPR, 2004). Due to its high K_{oc} value (15,415–75,900 L kg^{-1}), it is believed to have a high affinity to soils containing higher contents of organic C (Tomlin, 2003). While its degradation is rapid in aquatic environments, it is more stable in terrestrial environments. Quinoxyfen is poorly soluble and is tightly adsorbed to the soil sorption complex. Field studies have shown that quinoxyfen (as well as its residues) can accumulate in soils and due to its strong adsorption, it is not believed to pose a significant threat for groundwaters (CDPR, 2004).

3.1.8. Tebuconazole

Tebuconazole is a systemic fungicide which is highly effective for controlling soil-borne and foliar fungal pathogens. Its soil half-life ranges from 40 to 170 d depending on the amount of applied fungicide per ha (FAO, 1994). Its degradation is also significantly influenced by the organic C content because it exhibits a high affinity for SOM (Berenzen et al., 2005). The K_d value varies from 7.7 to 16.4 L kg^{-1}, and K_{oc} values range from 911 (silt) to 1251 L kg^{-1} (sandy loam soil), which again indicates that soils with higher clay content exhibit stronger sorption. Bending et al. (2007) have shown a similar K_d value (the average in sandy loam soils was 12.4 L kg^{-1}). The amount of tebuconazole adsorbed in the soil varies between 28 and 74% and is mainly concentrated in the top soil layer (0–15 cm) (FAO, 1994). Strickland et al. (2004) have found that the soil half-life of tebuconazole reaches 49 d in loamy sand soils. The value was probably influenced by a relatively high temperature and moisture combined with a low organic C content in the soil. Low organic C content contributes to decreased sorption and encouraging thus microbial degradation of the fungicide. Tebuconazole has a tendency to degrade more quickly in soils under vegetation cover. Vegetation contributes to higher microorganism diversity in the soil and hence supports fungicide degradation. As it was not detected in deeper soil layers, groundwater contamination should not occur. The degradation in water depends on humic acid or nitrate contents and the addition of these compounds to soils can thus increase its degradation (FAO, 1994). Tebuconazole was detected in headwater streams situated in an agricultural environment around Braunschweig, Lower Saxony, Germany, at a concentration of 9.1 μg L^{-1} (Berenzen et al., 2005) and hence fungicide concentrations in water should be monitored in river basins devoted to vineyards. Vineyards are usually placed at sloped areas which poses a risk of fungicide washing-off to streams and water supplies.

3.1.9. Mancozeb

Mancozeb is a contact fungicide used for treatment of a wide variety of crops and seeds. It is practically insoluble in water and most organic solvents (Kamrin, 1997). Mancozeb decomposes in the pH range of 5–9, at increased temperature and upon exposure to moisture and air. Furthermore, it degrades quickly by hydrolysis (USEPA, 2005). Mancozeb has low persistence in soils with a half-life of 1–7 d. It easily degrades to ethylenethiourea (ETU) which can persist in soils for 5–10 weeks (Kamrin, 1997) and due to its high solubility and mobility, ETU has the potential to migrate from soils to groundwater (USEPA, 2005). According to the report of the EC (2009), K_d values ranged from 7.3 to 11.7 L kg^{-1} and K_{oc} values from 363 to 2334 L kg^{-1}, depending on the soil characteristics. K_{oc} values indicate that mancozeb is moderately to slightly mobile in soils (IUPAC, Pesticide Properties Database).

3.1.10. Pyrimethanil

Pyrimethanil is a contact fungicide used to control grey mould (Rose et al., 2009). It has a high affinity for the soil solid phase and thus is not easily degraded. Up to 80% can be adsorbed in soils after 24 h (Vanni et al., 2003). The authors have also shown that the rate of disappearance of pyrimethanil is much faster in the top layer of the soil due to intensive photo- and microbial degradation. Pyrimethanil is practically immobile in soil profiles; therefore, it has a low potential for leaching into the groundwater. When it reaches surface water, pyrimethanil degrades rapidly and/or is moderately adsorbed to sediments (Tomlin, 2003).

3.2. Toxicity of synthetic organic fungicides

The toxicity of fungicides, classified according to the USEPA, is summarized in Table 4. As mentioned above, the intensive use of fungicides in vineyards may lead to the contamination of ground and surface waters and pose a toxicological risk for organisms in the surrounding ecosystems. Metalaxyl is nearly nontoxic to freshwater fish and it is slightly toxic to aquatic organisms (Kamrin, 1997; Shengwen and Weiping, 2008). Moderate toxicity to aquatic organisms has been observed for propiconazole, procymidone and vinclozolin (FAO, 2001; EC, 2003b; Martinovic et al., 2008), while azoxystrobin, cyprodinil and quinoxyfen are classified as highly to extremely toxic to aquatic invertebrates (USEPA, 1998; CDPR, 2004; PMRA, 2007; Warming et al., 2009). However, toxic concentrations of quinoxyfen are not expected to occur in aquatic environments due to its chemical properties (poor water solubility, high K_{oc} values, which indicate strong adsorption to soil) (CDPR, 2004). Dewez et al. (2005) and Verdisson et al. (2001) have observed adverse effects on aquatic plants in the presence of fludioxonil and pyrimethanil. Negative

Table 4
Toxicity of fungicides (adapted from Kamrin, 1997).

EPA toxicity class	Toxicity rating	Characteristic acute toxicity in experimental animals	Example
I	Highly toxic	Oral LD_{50}: 0–50 mg kg^{-1} Dermal LD_{50}: 0–200 mg kg^{-1} Inhalation LC_{50}: 0–0.2 mg L^{-1} Skin/eye irritation: severe	
II	Moderately toxic	Oral LD_{50}: >50–500 mg kg^{-1} Dermal LD_{50}: >200–2000 mg kg^{-1} Inhalation LC_{50}: >0.2–2.0 mg L^{-1} Skin/eye irritation: moderate	Tebuconazole
III	Slightly toxic	Oral LD_{50}: 500–5000 mg kg^{-1} Dermal LD_{50}: >2000–20,000 mg kg^{-1} Inhalation LC_{50}: >2.0–20 mg L^{-1} Skin/eye irritation: slight	Metalaxyl, fludioxonil, propiconazole, vinclozolin, cyprodinil, azoxystrobin, tebuconazole
IV	Practically nontoxic	Oral LD_{50}: >5000 mg kg^{-1} Dermal LD_{50}: >20,000 mg kg^{-1} Inhalation LC_{50}: >20 mg L^{-1} Skin/eye irritation: none	Pyrimethanil, propiconazole, vinclozolin, cyprodinil, azoxystrobin, mancozeb

effect (temporary inhibition of photosynthesis of grapevine fruiting cuttings) of fludioxonil and pyrimethanil applications has been also observed by Saladin et al. (2003) and Petit et al. (2008).

Considering fungicides' ability to accumulate and adsorb to soil particles, they can significantly influence soil organisms and ecosystems. Sukul (2006) has shown that metalaxyl application leads to a reduction of microbial biomass as well as of total N and organic C contents in soils. Furthermore, a stimulation of ammonifying bacteria growth, adverse effects on P mineralization and the decrease in soil pH that was caused by fungicide primary acid metabolites has been also observed (Monkiedje et al., 2007). A similar pattern has been found for propiconazole by Ekelund et al. (2000a,b). The authors showed that propiconazole at concentrations commonly used in agriculture (~600 mg L^{-1}) negatively affected flagellates and changed the composition of their species. On the contrary, the presence of tebuconazole (the same chemical class as propiconazole) in soils did not have any significant effect on soil microbial biomass and the bacterial community structure. However, it reduced dehydrogenase activity in soils low in SOM (Bending et al., 2007). Adverse effects were also observed for numbers of nitrifying bacteria, nitrogen-fixing bacteria, numbers of fungi and nitrogen concentrations in tebuconazole-treated soils (Cycoń et al., 2006). Toxicological tests showed that commercially available fungicides containing metalaxyl pose a risk to soil microorganisms (Monkiédjé et al., 2000). On the other hand, Monkiedje and Spiteller (2002b) have shown that metalaxyl application has also a positive effect on soil properties. The activity of phosphatases and β-glucosidase, mineralization and the availability of N (and of most plant nutrients) in soils was stimulated. On the other hand, metalaxyl application inhibited dehydrogenase activity and the availability of NO_3^-.

Most of the fungicides mentioned above do not present a toxicological risk to terrestrial wildlife (USEPA, 1998; Tomlin, 2003; CDPR, 2004), only vinclozolin exhibits low levels of acute risk to wildlife (USEPA, 2000). Also mancozeb and its degradation product ETU have been shown to be toxic for several animals (e.g., frogs), even at low concentrations (Harris et al., 1998; Shenoy et al., 2009) and can also affect the reproduction of some animals (Kamrin, 1997).

3.3. Contamination of vineyard soils with synthetic organic fungicides

The intensive application of synthetic organic fungicides has led to increased concentrations of these chemicals in vineyard soils, according to the periods of treatments. It is needed to point out that the vast majority of data about the contamination of vineyard soils with synthetic organic fungicides originates from Spain. Bermúdez-Couso et al. (2007) have shown that the total fungicide concentration in acid vineyard soils in the Ourense province reached 1124 μg kg^{-1}. Procymidone, metalaxyl, fludioxonil and cyprodinil appeared at higher concentrations in soils (up to 1124, 1002, 349 and 462 μg kg^{-1}, respectively). While, procymidone, fludioxonil and cyprodinil residues in soils were higher in autumn and winter, metalaxyl and penconazole residues were higher in spring indicating that the application time of the fungicides significantly influences their concentrations. A similar pattern has been found in Rías Baixas (Spain) by Rial-Otero et al. (2004). Tebuconazole, procymidone, cyprodinil, and fludioxonil were detected in autumn (1 month after the last application) at concentrations 12, 20, 260 and 991 μg kg^{-1}, respectively. In spring, metalaxyl, procymidone, tebuconazole and cyprodinil were also detected in soils at subquantitation levels; and only fludioxonil was detected at a concentration higher than its limit of quantification (52 μg kg^{-1}). Arias et al. (2005b) have also reported cyprodinil and fludioxonil presence in vineyard soils of the Galician province Pontevedra (northwestern Spain) at concentrations of 9 and 142 μg kg^{-1}, respectively. Arias et al. (2006) have also reported metalaxyl and penconazole presence in vineyard soils of the same region in spring at concentrations of 25 and 50 μg kg^{-1}, respectively.

3.4. Interactions between Cu and synthetic organic pesticides

Pateiro-Moure et al. (2007) studied the interactions between Cu and organic herbicides (paraquat, diquat and difenzoquat) in vineyard soils and observed that increased Cu concentrations can displace difenzoquat from adsorption sites and that Cu adsorption is reduced in the presence of the herbicides in soils. In another study, Arias et al. (2006) found that while the addition of Cu does not influence the sorption of metalaxyl onto soil, it increased the adsorption of penconazole through the formation of Cu-penconazole complexes with an increased affinity for soil colloids. The same effect has been observed for the adsorption of the commonly used herbicide glyphosate (Morillo et al., 2000). The application of glyphosate to vineyard soils possibly leads to the formation of Cu-glyphosate complexes (especially in acidic soils) which can leach through the soil profiles (Dousset et al., 2007). As mentioned before, the interactions of Cu with organic pesticides influence their behavior and toxicity. Jacobson et al. (2005) found that increased Cu concentrations in vineyard soils influence the fate and transport of diuron in vineyard soils through an alteration of microbiological populations or activities involved in their degradation. On the other hand, diuron was found to prevent Cu-induced decreases of chlorophyll contents in some plants (Teisseire et al., 1999).

3.5. Impact of synthetic organic fungicides on health

Although not sufficiently in many cases, negative effects of some pesticides on human health (neurologic dysfunctions and diseases, hypospadias, endocrine disruption and increases in the incidence of cancers) have been reported (Saracci et al., 1991; Blair et al., 1993; Garry et al., 2002; Kamel and Hoppin, 2004). Mancozeb and other ethylenebis(dithiocarbamate) fungicides (maneb, zineb, metiram etc.) are absorbed primarily dermally and are metabolized to ethylenethiourea (ETU) (USEPA, 1992). In general, these compounds rapidly pass from the gastrointestinal tract into the bloodstream and may accumulate in liver, thyroid and nervous system (Kamrin, 1997). Ethylenebis(dithiocarbamate) fungicides affect the thyroid gland and the lymphocyte genome (Steenland et al., 1997), enhance the production of the thyroid-stimulating hormone and probably cause higher prevalence of solitary nodules of workers working with mancozeb (Panganiban et al., 2004). Mancozeb is also probably hormonally active as an estrous cycle-disrupting fungicide (Farr et al., 2004). On the other hand, Colosio et al. (2007) have suggested that seasonal application of mancozeb may cause changes of some immune parameters, but does not pose a significant immunotoxic risk for vineyard workers (Colosio et al., 2007). Red itchy rash with blistering was noticed after the exposure of workers exposed to quinoxyfen (Chowdhury et al., 2001). To our best knowledge, studies focused on the risk of human exposure to other fungicides mentioned in this review are lacking; therefore, further investigations should be performed to evaluate possible risks for workers working with these chemicals. It is needed to point out that each fungicide commercially used is sold with a safety data sheet summarizing the possible risks and measures associated with its use.

4. Conclusions

Polluted agricultural soils present a serious agro-environmental concern. The contamination with organic and inorganic pollutants reduces the quality of the soil and the sustainability of the agroecosystem. Copper originating from the intensive application of Cu-based fungicides belongs to the most important contaminants of vineyard soils as its concentrations exceed European legislative limits in the majority of the studied vineyards. Despite the fact that Cu in soils is strongly immobilized by the soil sorption complex (i.e., SOM, Fe-, Mn-oxyhydroxides etc.), there has been evidences of Cu migrating through soil profiles in vineyards which poses thus an important risk for groundwater quality. This is especially true for acidic soils, tilled soils and soils affected by intensive erosion. Therefore, stabilization strategies that lower the intensity of erosion (such as the application of organic amendments and vegetation cover) are needed for decreasing Cu runoff from vineyard soils via wind and water erosion. There have been only rare cases of Cu phytotoxicity symptoms observed on vine plants, mostly due to their deep rooting. However, precautions are needed when other crops with shallow roots are subsequently grown on such contaminated soils. Copper concentrations should be then lowered to acceptable levels through various remediation procedures.

Synthetic organic fungicides belong to various chemical classes and it is thus difficult to generalize their behavior in soils. Their sorption, mobility, stability, degradability and toxicity differ due to their different chemical properties and are significantly influenced by soil properties. Some of these compounds have been detected in surface waters at concentrations higher than the EU regulatory limit and hence it can be assumed that their extensive use poses a risk for the environment and human health. Therefore, the choice of fungicides should be performed carefully in accordance with agriculture practices, physico-chemical characteristics of the soils and climatic and hydrogeological regimes in separate vine-growing regions. Additionally, soil amendments, such as organic matter application, can improve soil quality and reduce the risks associated with leaching of the fungicides. Sensitive approaches to pest control, such as the Integrated Pest Management, which is used to manage pest damage by the most economical means with the least possible hazard to humans and the environment, should be encouraged. New-generation fungicides should be practically nontoxic, except for the target organism, and upcoming research should evaluate the toxicity of synthetic organic fungicides and their residues to humans.

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Complexation between the fungicide tebuconazole and copper(II) probed by electrospray ionization mass spectrometry

Jana Jaklová Dytrtová[1]*, Michal Jakl[2], Detlef Schröder[1], Eva Čadková[2] and Michael Komárek[2]

[1]Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, v.v.i., Flemingovo náměstí 2, 166 10 Prague 6, Czech Republic
[2]Department of Agro-Environmental Chemistry and Plant Nutrition, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague–Suchdol, Czech Republic

Electrospray ionization mass spectrometry (ESI-MS) is used to probe the complex formation between tebuconazole (1) and copper(II) salts, which both are commonly used fungicides in agriculture. Experiments with model solutions containing 1 and $CuCl_2$ reveal the initial formation of the copper(II) species $[(1)CuCl]^+$ and $[(1)_2CuCl]^+$ which undergo reduction to the corresponding copper(I) ions $[(1)Cu]^+$ and $[(1)_2Cu]^+$ under more drastic ionization conditions in the ESI source. In additional experiments, copper/tebuconazole complexes were also detected in samples made from soil solutions of various origin and different amount of mineralization. The direct sampling of such solutions via ESI-MS is thus potentially useful for understanding of the interactions between copper(II) salts and tebuconazole in environmental samples. Copyright © 2011 John Wiley & Sons, Ltd.

Recently, we initiated a research project aimed towards the application of electrospray ionization (ESI) mass spectrometry (MS) for the characterization of complexes of coinage metals in the gas phase,[1,2] with particular attention being paid to nitrogen ligands.[3–9] In the course of these studies, we came across a possibly interesting cross-relation between copper and nitrogen compounds in crop science, which we considered being worth a more detailed investigation.

Specifically, the compound tebuconazole, i.e. racemic 1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl) pentan-3-ol (**1**), is a systemic triazole fungicide, which inhibits ergosterol biosynthesis. It is used for controlling soil-borne and foliar fungal pathogens. According to a classification by Kamrin,[10] tebuconazole is ranked among the pesticides slightly soluble in water ($0.036\,g{\cdot}L^{-1}$ at 20°C). Frequent application of tebuconazole leads to its accumulation in soils. The half-life of **1** in soils ranges from 40 to 170 days depending on soil characteristics (organic matter, clay content, etc.). Major degradation products in soils include triazolylacetic acid, hydroxytriazole, and triazolylalanine.[11] Degradation intermediates in water are *tert*-butyl alcohol and butyrate derivatives of **1**. Enteropathic recirculation occurs in intact animals.[12] The main metabolites include alcohols and carboxylic acids that indicate the oxidation of methyl group(s) of the *tert*-butyl moiety, and *tert*-butyl alcohol is one of the metabolites.[13]

Copper compounds are widely used inorganic fungicides. They can interact with other agrochemicals present in soils and thus influence their behavior in the environment, i.e., accumulation in soils, degradation, mobility, and stability.[14,15] Although both fungicides are usually applied on plants, they can be transported to the soil through washing-off or falling of plant material,[16] and this can subsequently cause risks for soil ecosystems, groundwater and surface water. There has been evidence that triazole fungicides can create complexes with various metals, including complexes of tebuconazole with copper(II).[17,18] Likewise, Arias *et al.*[14] pointed out the different behavior of free and complexed triazoles in soil environment in that the adsorption is increased in the presence of Cu due to the formation of binary complexes.

The aim of the present study is to investigate tebuconazole-copper interactions and possible changes of the oxidation state of Cu in its complexes with tebuconazole. The results can contribute to a better understanding of the behavior of agrochemicals in the environment and thereby assist in preventing risks associated with their application.

EXPERIMENTAL

The experiments were performed with a Finnigan LCQ Advantage ion-trap mass spectrometer (ThermoFinnigan, San Jose, CA, USA) fitted with an electrospray ionization source operated in positive and negative-ion mode.[19] Dilute solutions of tebuconazole and appropriate copper(II) salts in methanol/water (1:1 v/v) were introduced into the ESI source via a fused-silica capillary at a flow rate of $1.3\,mL{\cdot}h^{-1}$. Nitrogen was used as the nebulizer gas. The operating conditions were set as follows: spray voltage 5.0 kV, capillary voltage 105 V, heated capillary temperature 250°C, sheath gas flow rate and auxiliary gas flow rate 20–30 arbitrary units.

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* *Correspondence to:* J. Jaklová Dytrtová, Institute of Organic Chemistry and Biochemistry of the AS CR, v.v.i., Flemingovo náměstí 2, 166 10 Prague 6, Czech Republic.
E-mail: dytrtova@uochb.cas.cz

The ionization conditions in ESI are critically influenced by the settings of the capillary voltage and the tube lens offset. When large voltages are applied, multicollisional activation in the medium-pressure regions of the ion source leads first to desolvation and then to collision-induced dissociation (CID),[20,21] which in the case of copper(II) compounds, also includes redox reactions to copper(I) species.[22–25] Unless noted otherwise, we have used medium conditions which afford $\mathrm{Cu^I}$ and $\mathrm{Cu^{II}}$ species in comparable abundances. Mass spectra were recorded from *m/z* 50 to 2000. CID of mass-selected precursor ions was achieved by radio-frequency (RF)-excitation of the ions within the helium buffer gas present in the ion trap as the collision partner. The collision energy was optimized for each experiment and is expressed in terms of the manufacturer's normalized collision energy (%), where the range from 0 to 100% corresponds to a resonance excitation alternating current (*a.c.*) signal of 0–2.5 V (zero-to-peak) at the secular frequency of the ion of interest. The actual collision energies depend on the *m/z* values of the precursor ions, but conversion to an absolute energy scale is feasible via an empirical calibration,[24] for which we have applied the loss of pyridine from mass-selected (2,5-dimethylylbenzyl)pyridinium ion which leads to the phenomenological conversion $E_{\mathrm{coll}}(\mathrm{kJ{\cdot}mol^{-1}}) = (7.3 \pm 0.3) \bullet E_{\mathrm{coll}}(\%)$ for the ion-trap mass spectrometer used in the present experiments.[26]

The model solutions were prepared from $\mathrm{CuCl_2}$ salt (purum), tebuconazole, HPLC-grade methanol (all from Sigma-Aldrich, Prague, Czech Republic), and deionized water (>18MΩ). The stock solutions were equimolar in $\mathrm{CuCl_2}$ and tebuconazole (both $5{\cdot}10^{-4}\,\mathrm{mol{\cdot}L^{-1}}$) and diluted to the desired concentrations.

RESULTS AND DISCUSSION

Figure 1 shows a positive mode ESI mass spectrum of a dilute mixture of tebuconazole and $\mathrm{CuCl_2}$ in methanol/water. The major ions observed can be divided into four groups; note that all listed *m/z* values refer to the light isotopes (^{1}H, ^{12}C, ^{14}N, ^{35}Cl, and ^{63}Cu). (i) In several $\mathrm{Cu^{II}}$ complexes originated from the precursor metal salt, e.g., $[(\mathbf{1})\mathrm{CuCl}]^+$ (*m/z* 405) and $[(\mathbf{1})_2\mathrm{CuCl}]^+$ (*m/z* 712), the valence of the metal is maintained upon ESI. (ii) In contrast, the signals due to $[(\mathbf{1})\mathrm{Cu}]^+$ (*m/z* 370) and $[(\mathbf{1})_2\mathrm{Cu}]^+$ (*m/z* 677) demonstrate a reduction to $\mathrm{Cu^I}$. In the case of $[(\mathbf{1})_2\mathrm{Cu}]^+$, some fragmentation via loss of (presumably) isobutane is also observed, which leads to $[(\mathbf{1})_2\mathrm{Cu\text{-}C_4H_{10}}]^+$ (*m/z* 619). (iii) To a smaller, but yet non-negligible extent, the formation of dinuclear cluster ions, such as $[(\mathbf{1})\mathrm{Cu_2Cl_3}]^+$ (*m/z* 538) and $[(\mathbf{1})_2\mathrm{Cu_2Cl_3}]^+$ (*m/z* 845), is observed. In the higher mass range (not shown), some oligonuclear clusters are also observed in correspondingly low abundances, e.g., $[(\mathbf{1})_2\mathrm{Cu_3Cl_5}]^+$ (*m/z* 978), $[(\mathbf{1})_3\mathrm{Cu_3Cl_5}]^+$ (*m/z* 1285), and $[(\mathbf{1})_3\mathrm{Cu_4Cl_7}]^+$ (*m/z* 1418). In the vast majority of these cluster ions, copper is in the $\mathrm{Cu^{II}}$ state, which is consistent with a generation of these larger aggregates in the absence of energizing collisions. (iv) Last, but not least, a signal due to protonated tebuconazole, $\mathbf{1}\mathrm{H}^+$ (*m/z* 308), is observed, whose formation can be explained by the slightly acidic properties of the aqueous $\mathrm{CuCl_2}$ solutions. We note that under the conditions chosen, dicationic copper complexes are observed only in traces, e.g., a small signal due to $[(\mathbf{1})_4\mathrm{Cu}]^{2+}$ (*m/z* 645.5).

The formation of copper(I) ions might appear surprising, because it is commonly assumed that the oxidation state of metal compounds is maintained in ESI.[27,28] In fact, redox reactions between copper(II) and 1,2,4-triazoles have been reported in which copper serves as an electron acceptor,[29] and triazoles seem to generally stabilize low oxidation states of copper because they are also used for the protection of metallic copper surfaces against corrosion.[30] However, it appears much more likely that the reduction occurs in the electrospray process itself.[20,22–25] In order to address the generation of the copper(I) species in more detail, several ions were mass-selected and submitted to CID. Thus, mass-selected $[(\mathbf{1})\mathrm{Cu}]^+$ (*m/z* 370) shows loss of water as predominant fragmentation channel, not unexpected for a tertiary alcohol such as **1**. Mass-selected $[(\mathbf{1})\mathrm{CuCl}]^+$ (*m/z* 405) undergoes loss of HCl to afford $[(\mathbf{1}\text{-H})\mathrm{Cu}]^+$ (*m/z* 369), in which the formal oxidation state $\mathrm{Cu^{II}}$ is maintained. The biscoordinated $\mathrm{Cu^I}$ species $[(\mathbf{1})_2\mathrm{Cu}]^+$ (*m/z* 677) shows preferential losses of the neutral ligand to afford $[(\mathbf{1})\mathrm{Cu}]^+$. In contrast, the $[(\mathbf{1})_2\mathrm{CuCl}]^+$ ion (*m/z* 712) expels a chlorine radical upon CID thereby affording $[(\mathbf{1})_2\mathrm{Cu}]^+$ along with a minor loss of neutral **1** to produce $[(\mathbf{1})\mathrm{CuCl}]^+$. Observation of chlorine-atom loss provides a straightforward rationale for the occurrence of the redox process and has precedence in the gas-phase chemistry of CuCl* complexes.[24,25] Figure 2 summarizes the

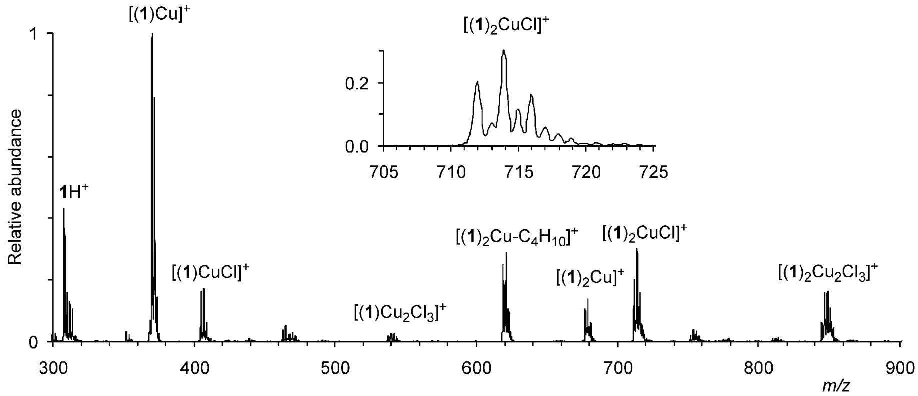


Figure 1. Positive mode ESI mass spectrum in the range from *m/z* 300 to 900 of an equimolar mixture of tebuconazole (**1**) and $\mathrm{CuCl_2}$ (both $5{\cdot}10^{-4}\,\mathrm{mol{\cdot}L^{-1}}$) methanol/water (1:1). As an example, the inset on the top shows the isotope pattern of the $[(\mathbf{1})_2\mathrm{CuCl}]^+$ complex on an expanded mass scale.

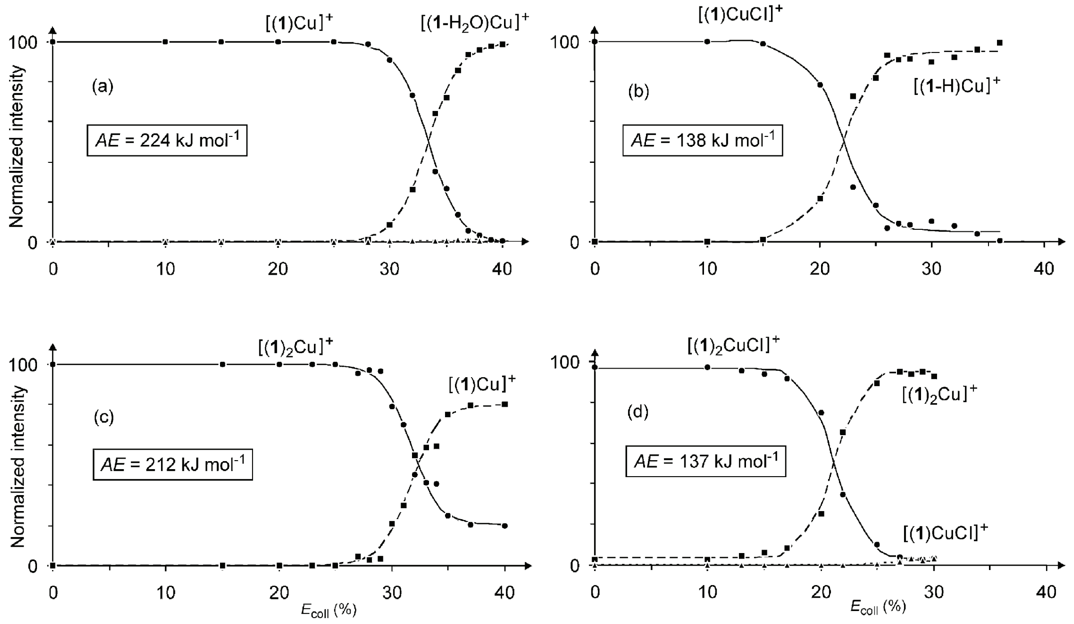


Figure 2. Energy-dependences of the product ion abundances for (a) $[(\mathbf{1})Cu]^+$, (b) $[(\mathbf{1})CuCl]^+$, (c) $[(\mathbf{1})_2Cu]^+$, and (d) $[(\mathbf{1})_2CuCl]^+$ derived from independent CID experiments with the mass-selected ions.

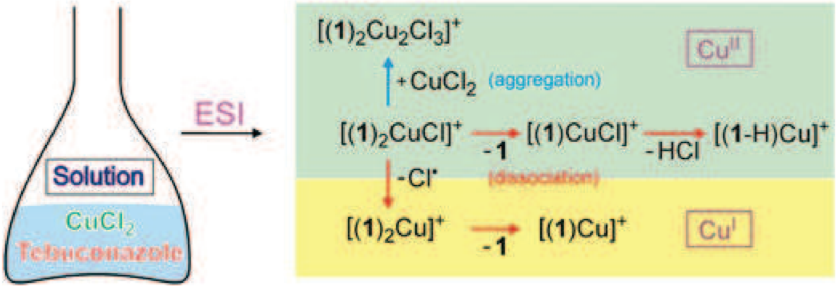
energy-dependences of the fragmentation channels for these four representative ions. Consistent with the fragmentation behavior described above and the general gas-phase chemistry of ligated copper ions,[1,2] the appearance energies for the fragmentation of the Cu^I species $[(\mathbf{1})Cu]^+$ and $[(\mathbf{1})_2Cu]^+$ are significantly larger than those for the loss of atomic chlorine from the Cu^{II} compounds $[(\mathbf{1})CuCl]^+$ and $[(\mathbf{1})_2CuCl]^+$.

With this information, we can propose a simple scheme for the generation of the various ions upon ESI of the tebuconazole/$CuCl_2$ solution in methanol/water (Scheme 1). Initially upon sampling, intact copper(II) complexes with tebuconazole evolve from solution with $[(\mathbf{1})_2CuCl]^+$ as the major species. Because the concentration of the non-volatiles is increased in the spray process,[31,32] some of these species undergo clustering to binuclear species. Under harsher conditions of ionization, the metal is reduced from Cu^{II} to Cu^I at the stage of $[(\mathbf{1})_2CuCl]^+$ and all other major ions observed can be accounted for by subsequent losses of closed-shell fragments.

The ratio between the ions from the copper(I) and copper (II) manifolds can be varied by appropriate adjustment of the relevant potentials in the ESI source. Figure 3 shows the influences of the capillary voltage and the tube lens offset for the two most abundant representatives in the spectra, i.e., $[(\mathbf{1})Cu]^+$ for the Cu^I species and $[(\mathbf{1})_2CuCl]^+$ for the Cu^{II} manifold. In these experiments, one of the values was kept fixed while the other was changed in steps of 5 V. Both diagrams show the preference for Cu^{II} species at low voltage settings (= soft ionization conditions) with a switch to the reduced Cu^I ions under harder conditions.

Having understood the chemistry occurring in the ESI process and having achieved means to modify the ratio between Cu^I and Cu^{II} species, we can now exploit these results in the detection of possible tebuconazole/copper complexes in real samples in a direct mass spectrometric assay, i.e., without a prior workup of the samples. The latter aspect is of particular importance for an evaluation of the role of possible association between tebuconazole and copper species in environmental samples, because conventional workup for the detection of organic fungicides via extraction and subsequent chromatographic separation is likely to destroy the copper complexes eventually present in the crude samples.

At first, the concentration of the analytes was reduced by dilution in order to probe the sensitivity of direct sampling



Scheme 1. Degradation pathways of copper/tebuconazole complexes formed upon ESI and transition from the copper(II) to the copper(I) manifold.

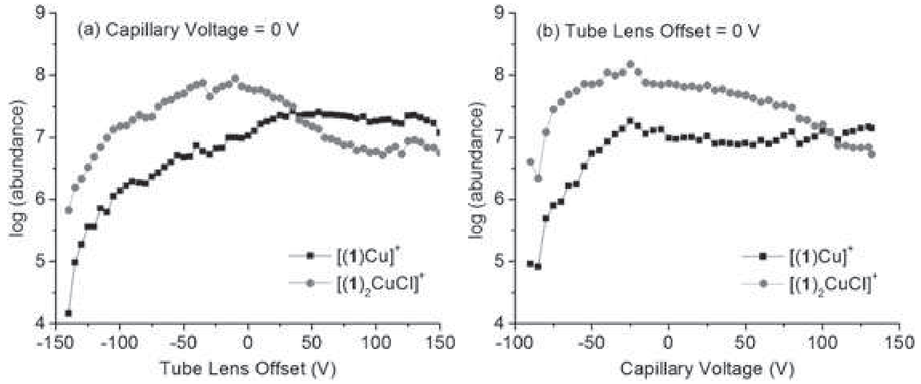


Figure 3. Dependences of the abundances (in logarithmic scale) of the most abundant Cu^I and Cu^{II} species from (a) the capillary voltage and (b) the tube lens offset.

via ESI-MS. As shown in Fig. 4, even concentrations below 10^{-6} mol·L^{-1} give clearly detectable signals of the tebuconazole/copper complexes. The measurements were then repeated in a 1:1 v/v mixture of methanol and tap water as a solvent in order to probe the effect of modest mineralization. Likewise, a representative soil solution was used in order to probe possible matrix effects. A sample of soil solution was collected from pot cultivation experiments with willow planted on Cambisol (soil classification according to the FAO World Reference Base for Soil Resources) with a low concentration of metals (Cu 6.5·10^{-8} mol·L^{-1}, Cd 1.3·10^{-8} mol·L^{-1} and Pb 5·10^{-9} mol·L^{-1}) via suction cups.[33] A typical soil solution is rich in organic matter, which is able to fix relatively high amounts of metal cations.[34] In both cases, the sensitivity is significantly lowered as can be seen by the elevated noise level in Fig. 5.

Finally, we have studied a soil solution sampled from a forest Spodosol with alder plantation (with content of organic matter lower than in the soil solution from given Cambisol) contaminated with copper in the long term (1.8·10^{-6} mol·L^{-1}),[35] added **1**, but omitted the addition of copper(II). As demonstrated in Fig. 6, under these conditions distinct signals due to the expected copper complexes of tebuconazole can also be identified. The major additional signals are due to alkali complexes of **1**, which simply can be ascribed to the mineral contained in the soil solution. The organic matrix in the sample obviously does not prevent the detection of the complexes between copper and tebuconazole.

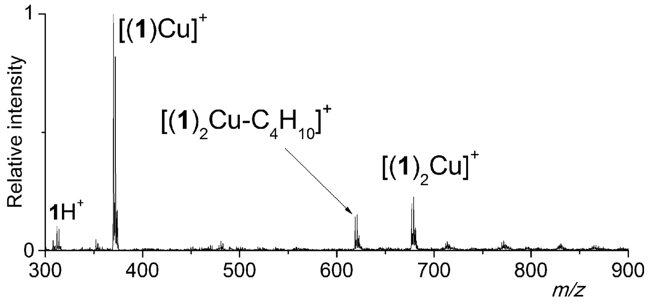


Figure 4. Positive mode ESI mass spectrum in the range from *m/z* 300 to 900 of an equimolar mixture of tebuconazole (**1**) and $CuCl_2$ (both 5·10^{-7} mol·L^{-1}) in methanol/water (1:1).

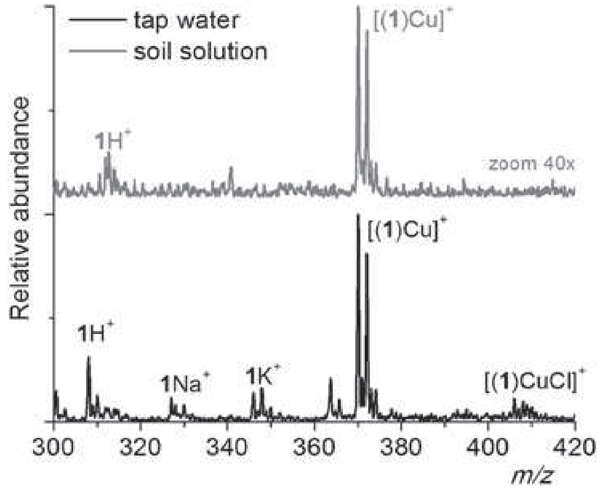


Figure 5. Positive mode ESI mass spectra in the *m/z* range from 300 to 420 of an equimolar mixture of tebuconazole (**1**) and $CuCl_2$ (both 5·10^{-5} mol·L^{-1}) tap water and soil solution.

CONCLUSIONS

Electrospray ionization mass spectrometry confirmed the high tendency of copper(II) ions to form complexes with the fungicide tebuconazole **1**, where the [(**1**)Cu]$^+$ and [(**1**)$_2$Cu]$^+$ ions are particularly well suited for the detection of mixed copper/tebuconazole species. Additional collision-induced dissociation experiments reveal that the reduction from copper(II) to copper(I) in these two complexes happens in the course of the electrospray process and does not take place in solution. Interestingly, the copper/tebuconazole complexes can be detected not only in idealized laboratory solutions using pure ingredients, but also in samples made from soil solutions of various origins and of different compositions. These findings are important from an agro-environmental perspective. The formation of tebuconazole-Cu complexes, e.g., in vineyard soils, where these fungicides are commonly applied, is an important factor influencing

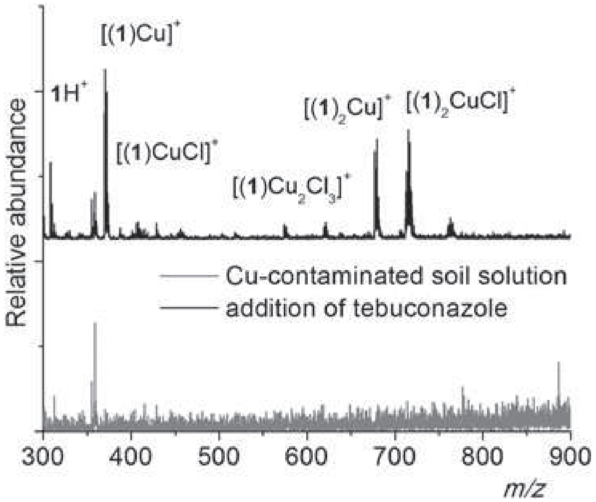


Figure 6. Positive mode ESI mass spectrum in the *m/z* range from 300 to 750 of a soil solution (from forest soil) naturally contaminated with Cu (ca. $0.9 \cdot 10^{-6}\,\text{mol} \cdot \text{L}^{-1}$ after dilution with methanol) before (bottom, grey) and after addition of tebuconazole (**1**) to final concentration $5 \cdot 10^{-5}\,\text{mol} \cdot \text{L}^{-1}$.

the behavior of both these chemicals. The complexes are likely to have sorption properties different from those of tebuconazole or Cu alone which will affect the (bio)degradability and toxicity. By parallel analytical methods we will try to determine to what extent the direct mass spectrometric sampling can also be used for quantitative studies of the interaction between copper(II) salts and tebuconazole, which are among the most commonly used fungicides in modern agriculture.

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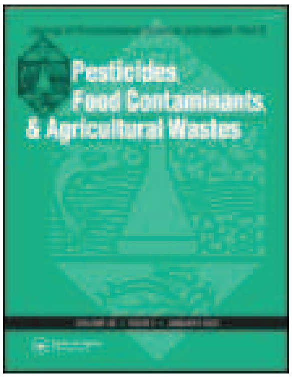

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Eva Čadková [a] , Michael Komárek [b] , Regina Kaliszová [a] , Věra Koudelková [c] , Jiří Dvořák [d] & Aleš Vaněk [e]

[a] Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Prague, Czech Republic

[b] Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic

[c] Department of Physics Education, Charles University in Prague, Prague, Czech Republic

[d] Department of Probability and Mathematical Statistics, Charles University in Prague, Prague, Czech Republic

[e] Department of Soil Science and Soil Protection, Czech University of Life Sciences Prague, Prague, Czech Republic

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Sorption of tebuconazole onto selected soil minerals and humic acids

EVA ČADKOVÁ[1], MICHAEL KOMÁREK[2], REGINA KALISZOVÁ[1], VĚRA KOUDELKOVÁ[3], JIŘÍ DVOŘÁK[4] and ALEŠ VANĚK[5]

[1]*Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Prague, Czech Republic*
[2]*Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Prague, Czech Republic*
[3]*Department of Physics Education, Charles University in Prague, Prague, Czech Republic*
[4]*Department of Probability and Mathematical Statistics, Charles University in Prague, Prague, Czech Republic*
[5]*Department of Soil Science and Soil Protection, Czech University of Life Sciences Prague, Prague, Czech Republic*

The aim of the present study was to investigate tebuconazole sorption on common soil minerals (birnessite, ferrihydrite, goethite, calcite and illite) and humic acids (representing soil organic matter). Tebuconazole was used (i) in the commercial form Horizon 250 EW and (ii) as an analytical grade pure chemical. In the experiment with the commercially available tebuconazole, a significant pH-dependent sorption onto the oxides was observed (decreasing sorption with increasing pH). The highest sorption was found for ferrihydrite due to its high specific surface area, followed by humic acids, birnessite, goethite and illite. No detectable sorption was found for calcite. The sorption of analytical grade tebuconazole on all selected minerals was significantly lower compared to the commercial product. The sorption was the highest for humic acids, followed by ferrihydrite and illite and almost negligible for goethite and birnessite without any pH dependence. Again, no sorption was observed for calcite. The differences in sorption of the commercially available and analytical grade tebuconazole can be attributed to the additives (e.g., solvents) present in the commercial product. This work proved the importance of soil mineralogy and composition of the commercially available pesticides on the behavior of tebuconazole in soils.

Keywords: Adsorption, fungicide, humic acids, soil minerals, tebuconazole.

Introduction

Tebuconazole [(RS)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-pentan-3-ol] is a systemic triazole fungicide which is used for controlling soil-borne and foliar fungal pathogens of many crop plants. Even though the fungicide is applied on plants, it can reach the soil through washing-off or falling of plant material[1] and thus can pose a risk for soil ecosystems, groundwater and water supplies.[2]

Adsorption is one of the key processes that influence the concentrations of pesticides in soil and soil solution and their movement through the soil profile and thus their potential for leaching into the groundwater.[3–6] Adsorption is mainly influenced by physico-chemical characteristics of the pesticide and soil properties.[4] In general, soil organic matter (SOM) is considered to be one of the most important factors influencing fungicide sorption.[7–9] Andrades et al.[10] have described the adsorption by both SOM and clays (with a predominant role of SOM). This assumption is supported by Monkiedje and Spiteller.[11] However, other authors have shown a more significant role of clay minerals and other soil components (e.g., Fe-, Mn-oxyhydroxides) in fungicide sorption.[12–14]

Tebuconazole is mainly adsorbed in the top soil layer (0–15 cm);[15] nevertheless, it has been also detected in headwater streams situated in an agricultural environment at concentrations reaching 9 μg L^{-1}.[16] Additionally, sorption processes decrease tebuconazole availability to microorganisms, degradability and thus enhance its accumulation in soils.[17] This highlights the importance of a detailed knowledge about the behavior of tebuconazole in soils.

Although some studies on tebuconazole sorption in soils have been published, to our best knowledge there is no study describing tebuconazole sorption onto separate soil minerals and SOM. These phases are the most abundant components in soils and represent important constituents of the soil sorption complex. Therefore, they can

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Address correspondence to Michael Komárek, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Praha 6, Czech Republic; E-mail: komarek@af.czu.cz; cadkovae@af.czu.cz
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significantly influence fungicide sorption and possible accumulation.

Most of the studies focused on tebuconazole sorption were performed using analytical grade tebuconazole despite the fact that the pure form is not used in agriculture. Commercially available products contain additives that support fungicide effectiveness and simplify its application. It must be pointed out that these additives in pesticide formulations can significantly influence the sorption mechanisms and subsequent behavior in soils.[18,19]

The aim of this study is thus to describe tebuconazole sorption onto various common soil minerals (birnessite, calcite, goethite, ferrihydrite, illite) at pH values 4.0, 5.5, 7 and to humic acids (representing SOM). Furthermore, the sorption of the commercial tebuconazole-containing fungicide Horizon 250 EW will be compared with analytical grade tebuconazole.

Materials and methods

Minerals and chemicals

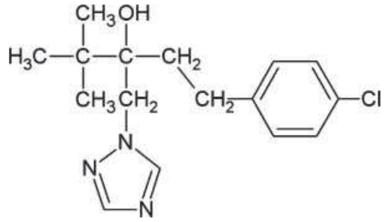
Two-line ferrihydrite and goethite were synthesized according to the procedure described by Schwertmann and Cornell,[20] birnessite by McKenzie[21] and calcite by Vaněk et al.[22] The prepared minerals were confirmed by XRD and VMP (voltammetry of particles) analyses. Pure illite was prepared from the clay standard IMt-1 (Cambrian shale, Silver Hill, Montana, USA) supplied by The Source Clays Repository (Purdue University, Indiana, USA). Illite preparation included grounding and separation using gravity sedimentation followed by centrifugation. Humic acids were purchased from Sigma-Aldrich (United States). The main characteristics of the selected soil minerals are summarized in Table 1.

Table 1. Main characteristics of synthetic minerals used in this study.

Mineral	*Formula*	*Specific surface area (m² g⁻¹)*
Birnessite	$K_4Mn_{14}O_{27}.9H_2O$	35.4–36.2[23, 24]
Calcite	$CaCO_3$	4.8–5.8[25, 26]
2-Line Ferrihydrite	$Fe_5HO_8{\cdot}4H_2O$	200−320[20]
Goethite	α-FeOOH	20[20]
Illite	$(K,H_3O)Al_2(Si,Al)_4O_{10}(OH)_2$	11[27]

Analytical grade tebuconazole was supplied by the Institute of Industrial Organic Chemistry, Warsaw (Poland) with a purity 99.9 ± 0.1 %. The main physico-chemical properties of tebuconazole are shown in Table 2. The commercially available tebuconazole-containing fungicide Horizon 250 EW was supplied by Bayer CropScience (Germany). All other chemicals were of analytical grade and supplied by Chromservis (Czech Republic).

Table 2. Physico-chemical properties of tebuconazole according to IUPAC (Pesticide Properties Database).[28]

Chemical name	(RS)-1-*p*-chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol
CAS Number	107534-96-3
Chemical class	Triazoles
Formula	
Molecular mass	307.8
Water solubility (g L⁻¹)	0.036 (at 20°C)
Melting point (°C)	105
log K_{ow}	3.7 (at 20°C)
K_{oc} (L kg⁻¹)	769
Degradation in soils (d)	55.8–365

Sorption experiment

Sorption experiments were performed using the batch equilibrium approach. Analytical grade tebuconazole stock solutions at concentration of 1g L^{-1} were prepared using methanol and kept in dark at 5°C. Aqueous solutions of tebuconazole (50–175 mg L^{-1}) were prepared by adding the appropriate volume of stock tebuconazole solutions to 0.01 M $CaCl_2$ (as the background electrolyte). The background electrolyte was chosen due to its wide use in other studies performing similar experiments[25,29,30] and was recommended by the OECD Guideline 106 for sorption experiments.[31] Methanol was subsequently removed using a rotary evaporator (Rotavapor R-215, Büchi, Switzerland). Aqueous solutions of the commercial product Horizon 250 EW (50–175 mg L^{-1}) were prepared by dissolving the appropriate amounts in 0.01 M $CaCl_2$.

Soil minerals and humic acids (ferrihydrite 0.39 g L^{-1}, goethite 7.44 g L^{-1}, birnessite 2.77 g L^{-1}, illite 2.00 g L^{-1}, calcite 7.44 g L^{-1} and humic acids 1.00 g L^{-1})[25,32,33] were equilibrated in 0.01 M $CaCl_2$ for 48 hours. Mineral suspensions were adjusted to pH 4, 5.5 and 7 by adding HCl or $Ca(OH)_2$. Some authors[32,33] showed that pH can markedly influence pesticide sorption. Hence this study also describes tebuconazole sorption at different pH values. The pH value of the calcite and humic acids suspensions were not adjusted because of their high pH-buffering capacity. The pH of the experimental solutions at equilibrium with the humic acids was 3.3 and with calcite 7.3. A volume of 10 mL of tebuconazole solution at desired concentration was added to 40 mL of mineral/humic acids suspensions. Samples were shaken for 24 hours (GFL 3017 orbital shaker, Germany), after which they were centrifuged for 10 min at 7000 rpm (Hettich Universal 30 RF centrifuge, Germany). Previous kinetic experiment showed that 24 hours was long enough to reach sorption equilibrium (data not shown). The supernatant was extracted by the organic solvent ethyl acetate by shaking the mixture for 1 h. An appropriate volume of ethyl acetate was removed to vials and analyzed using GC/MS (Agilent Technologies 6890N/5975, USA). The experiments were performed in triplicates and in the dark.

The amount of tebuconazole sorbed onto the minerals/humic acids was calculated as follows:

$$S = \frac{(C_i - C)}{m} \times V \tag{1}$$

where S is the sorbed concentration (mmol g^{-1}), C_i is tebuconazole initial concentration in solution (mmol L^{-1}), C is the aqueous equilibrium concentration (mmol L^{-1}), V is the volume of the liquid phase (L) and m is the mass of minerals/humic acids in the suspension (g).

The adsorption isotherms were created using different equations. The experimental data fitted with: (i) the Langmuir isotherm following equation:

$$S = S_{\max} \times \frac{KC}{1 + KC} \tag{2}$$

where S is the sorbed concentration (mmol g^{-1}), $S_{\max}$ is the maximum sorbed concentration (mmol g^{-1}), C is the aqueous equilibrium concentration (mmol L^{-1}), K is the Langmuir binding-strength coefficient (L $mmol^{-1}$); (ii) the sigmoidal Langmuir model following equation:

$$S = S_{\max} \times \frac{KC}{1 + KC + \frac{s}{C}} \tag{3}$$

where S, $S_{\max}$, C, K are the same as in Equation 2 and s is a constant related to the limitation of adsorption (mmol L^{-1}); and (iii) the Freundlich isotherm following equation:

$$S = K_f \times C^n \tag{4}$$

where S is the sorbed concentration (mmol g^{-1}), C is the aqueous equilibrium concentration (mmol L^{-1}), constants K_f (L g^{-1}) and n (dimensionless) characterize the adsorption capacity and intensity. The Langmuir and the Freundlich isotherms were created according to Bolster and Hornberger.[34] A Microsoft Excel spreadsheet developed by these authors was used to obtain isotherm parameters (through the sum of least squares) and model efficiency (goodness of fit). The sigmoidal Lagmuir was fitted to the data through least-squares using Mathematica 7 (software for symbolic and numeric computation).[35]

Results and discussion

Sorption experiments using analytical grade tebuconazole

Figure 1 summarizes results about the sorption of tebuconazole (analytical grade and commercial) onto separate minerals and humic acids. The adsorption isotherms describing tebuconazole sorption on these minerals exhibit preferentially the S-type shape (characterized by the sigmoidal Langmuir model) with exception for illite and humic acids, which were described best by the Langmuir isotherm. The sorption of analytical grade tebuconazole was pH-independent and the highest sorption was observed on ferrihydrite (0.028 mmol g^{-1}), followed by illite (0.009 mmol g^{-1}) and almost negligible for goethite and birnessite (Fig. 1). These results are in a good agreement with Clausen and Fabricius,[32] where insignificant adsorption of nonionic pesticides onto ferrihydrite and goethite was observed. The absence of a pH effect on tebuconazole sorption can be explained by the non-charged character of the molecule. Although, lower pH values (2.5) can lead to a positive charge of nonionic fungicides, which are more attracted to the negative charge of soil colloids;[36] however, such pH values are not realistic in natural soil environments.

The low sorption of azole-based fungicide (e.g., tebuconazole, penconazole) onto illite has been already reported[37] and no sorption of tebuconazole onto calcite was observed as expected.[25,38] It is most probably caused by the small specific surface area of calcite and possibly due to the predominant occurrence of neutral sites at pH 7.[39] However, there has been evidence in literature[25] of a weak sorption of anionic pesticides onto calcite (which is not the case here), depending on electrolyte concentration.

The affinity of analytical grade tebuconazole for humic acids was much higher compared to all the selected soil minerals (0.089 mmol g^{-1}). The experimental data of tebuconazole sorption onto humic acids fit well the Langmuir isotherm (Fig. 1). In general, humic acids (and SOM) act as efficient sorbents for pesticides compared to soil minerals, including oxides.[30,33] Humic acids, representing SOM, thus seem to be one of the main factors influencing tebuconazole sorption in soils. The importance of SOM and highly decomposed organic material as the most significant soil components for azole-based fungicides sorption in soils has been already documented.[1,14,40] On the other hand, Singh[41] presented that soil organic matter content was not the only factor that influences the sorption of triazole fungicides and pointed out the importance of clay minerals as well. However, our data about tebuconazole sorption onto illite disagree with such conclusions. As mentioned before, pH is a critical parameter influencing only the sorption of ionic pesticides onto SOM,[30,33] which is not the case in our study (Fig. 1). Another important factor influencing the results of sorption experiments is the type and strength of the electrolyte.[39,42] The addition of $CaCl_2$ even at low

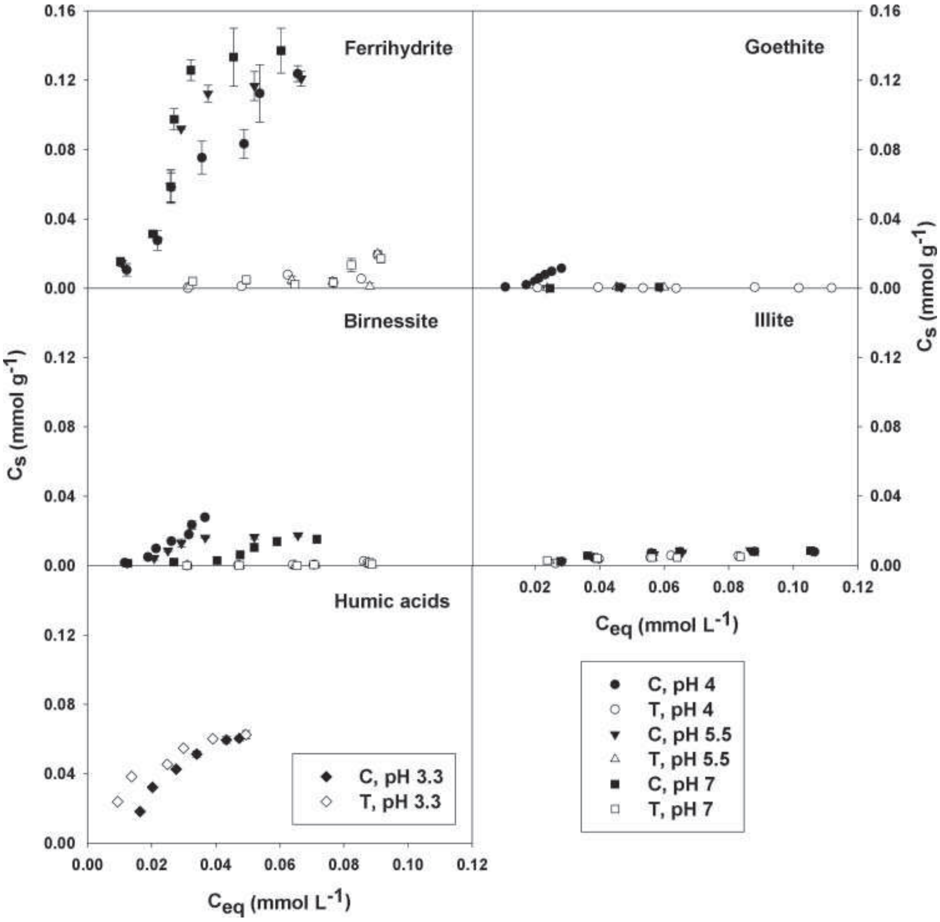


Fig. 1. Tebuconazole sorption onto selected soil minerals and humic acids at different pH values (with the exception of humic acids where the pH value was not adjusted), C_s-sorbed tebuconazole concentration at equilibrium; C_{eq}-final solute tebuconazole concentration at equilibrium; symbols represent: C-commercial formulation, T-analytical grade tebuconazole. Values shown are means ± SD (n = 3).

concentrations to the solution can reduce pesticide adsorption.[32] However, as mentioned before, we chose 0.01 M $CaCl_2$ as an electrolyte used in several other studies for comparative purposes.[25,29–31]

Sorption experiments using commercially-available tebuconazole

The sorption of tebuconazole from the commercial product Horizon 250 EW onto ferrihydrite, goethite and birnessite was significantly higher compared to analytical grade tebuconazole (Fig. 1). The adsorption isotherms describing tebuconazole sorption on these minerals exhibit preferentially the S-type shape (characterized by the sigmoidal Langmuir model), which can be the result of cooperative sorption of the organic additives, used as solvents and crystallization inhibitors (N,N-dimethyldecanamide) present in the commercial mixture, and tebuconazole. Organic additives, such as surfactants, are preferentially adsorbed onto mineral surfaces and thus enhance pesticide sorption.[43] In general, non-polar organic compounds, such as tebuconazole, have a low affinity for clay minerals. When the surface is covered by these compounds, other organic molecules are adsorbed more easily.[19,44]

The highest sorption of tebuconazole from the commercial product was found for ferrihydrite at pH 4 (0.202 mmol g^{-1}). With increasing pH, tebuconazole sorption decreased. These results are in a good agreement with other studies.[32] For example, the sorption of ionic pesticides (e.g., mecoprop, 2,4-D and bentazone) on ferrihydrite is higher at lower pH values with maximum adsorption close to the pK_a values of the acids.[32] Jia et al.[29] have also reported higher sorption of some triazoles onto 2-line ferrihydrite (up to 120 mg kg^{-1}) even at pH values close to 7.

Goethite showed an ability to adsorb commercial tebuconazole only at pH 4 (0.015 mmol g^{-1}). Similar results (decreasing pesticide sorption on goethite with increasing pH) were presented in Iglesias et al.[45] Similarly, the adsorption of organic acids onto goethite was studied and a similar trend in decreasing sorption on goethite with increasing pH was found.[46] In our experiment, tebuconazole adsorption onto goethite was significantly lower (one order of magnitude) compared to ferrihydrite. The same sorption trend onto ferrihydrite and goethite has been reported for ionic pesticides.[32] It is probably mainly caused by the higher crystallinity of goethite and the different specific surface areas (200−320 m^2 g^{-1} for ferrihydrite and 20 m^2 g^{-1} for goethite).[20] Sorption onto birnessite (0.038 mmol g^{-1}) was also more pronounced at lower pH and commercial tebuconazole adsorption was 10-fold higher compared to the analytical grade tebuconazole. The pH-dependent sorption behavior of tebuconazole from the commercially available fungicide can be thought as indirect; higher sorption observed for lower pH values indicates that the organic additive was retained at mineral surfaces and subsequently created sorption sites for tebuconazole.

Accordingly, illite showed a two-fold higher tebuconazole sorption (0.024 mmol g^{-1}) when applied in its commercial form. Similar results for penconazole were observed, including a significant increase of fungicide sorption onto illite after the addition of organic surfactants.[37] The experimental data of tebuconazole sorption onto illite fitted well the Langmuir isotherm. Tebuconazole sorption onto calcite was insignificant as in the case of analytical grade tebuconazole.

The sorption of commercial tebuconazole onto humic acids was again much higher compared to all selected minerals (except for ferrihydrite). The isotherm fit well the Freundlich model. The apparent lower affinity of commercial tebuconazole for the humic acids (compared to analytical grade; Fig. 1) was probably caused by its competition with organic additives for the sorption sites at low concentrations. The value of the Freundlich coefficient *n* was higher than 1, indicating a strong competition of water and/or additive molecules for adsorption sites at low fungicide concentrations.[10] The significant role of SOM in the sorption of commercial azoles was also reported.[19] In our study, the only exception was tebuconazole sorption onto ferrihydrite, which was higher compared to humic acids. It is probably caused by the large specific surface area, which helps to attract the organic additives contained in the commercial product enhancing thus tebuconazole sorption.

As mentioned earlier, the differences in sorption of the commercially available and analytical grade tebuconazole can be attributed to the additives (e.g., N,N-dimethyldecanamide) present in the commercial product that support fungicide effectiveness and simplify its application. Tebuconazole has a high affinity to organic compounds and the additives are mainly of organic origin. Sánchez-Martín et al.[37] supported this idea in their study where they compared the sorption ability of natural minerals and minerals modified with organic surfactants. The adsorption efficiency of natural minerals was much lower compared to the modified minerals. Sorption efficiency of azoles onto such modified clay minerals can be thus increased up to 133-times due to the high organic carbon content of organic surfactants.[47] The adsorbed amount of organic matter derived from the surfactant also differs with clay type.[37] Similar conclusions can be drawn from our study, where the additives positively influenced tebuconazole sorption onto the minerals. The amount of the adsorbed additive also differs depending on the type/specific surface area of minerals. This fact helps to explain the different tebuconazole sorption behavior on minerals: ferrihydrite > birnessite > goethite > illite > calcite. It is thus needed to point out that the additives in pesticide formulations can significantly influence the sorption mechanisms and behavior of the pesticide in soils. The minerals used in this study were pure, i.e., different from the phases found in natural environments. It is commonly accepted that “natural” minerals in soils are coated by various compounds, e.g., organic matter, oxides etc. and thus can have different sorption abilities.

The importance of soil composition and the presence of Cu-based fungicides on azole (penconazole) sorption has already been presented.[19] Soils higher in SOM with increased copper concentrations depicted higher sorption ability, especially when the fungicide was used as a commercial product. Also our results showed the importance of soil composition for predicting tebuconazole behavior in soil profiles and the possible risks for groundwater and water supplies and will be important for upcoming studies concerning tebuconazole sorption in real soils.

Conclusions

This model study confirmed that the retention of tebuconazole in soils is mainly controlled by soil organic matter content and mineralogical composition. Tebuconazole sorption was significantly higher when it was used from a commercial product containing N,N-dimethyldecanamide as solvent and crystallization inhibitor. From the minerals studied, the highest sorption was found for ferrihydrite due to its large specific surface area, followed by birnessite, illite and goethite. There was no evidence of tebuconazole sorption onto calcite. The different sorption behavior (including a pH-dependence) of the commercially available and analytical grade tebuconazole can be attributed to the additives present in the commercial product, which highlights the importance of the commercial fungicide compositions. Additionally, soil organic matter (represented by humic acids) strongly influences tebuconazole sorption in soils, regardless of its form (analytical grade or commercial product). Our data show that soil composition as well as soil pH can markedly influence the sorption of tebuconazole in commercial formulations, its accumulation or possible transport to surface and/or groundwater. In conclusion, our results are enlarging the knowledge about the environmental chemistry of azole-based pesticides and will be applicable to upcoming sorption experiments involving soils.

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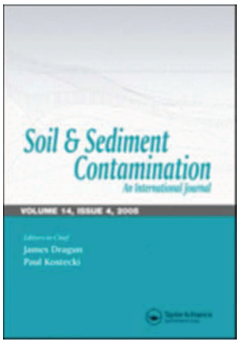

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Tebuconazole sorption in contrasting soil types

Eva Čadková[a*], Michael Komárek[b], Regina Kaliszová[a],
Aleš Vaněk[c], Miluše Balíková[a]

[a] Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, Praha 6 – Suchdol, 165 21, Czech Republic (cadkovae@af.czu.cz; kaliszova@af.czu.cz; balikova@af.czu.cz)
[b] Department of Environmental Geosciences, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, Praha 6 – Suchdol, 165 21, Czech Republic (komarek@fzp.czu.cz)
[c] Department of Soil Science and Soil Protection, Czech University of Life Sciences Prague, Kamýcká 129, Praha 6 – Suchdol, 165 21, Czech Republic (vaneka@af.czu.cz)

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* Corresponding author: tel. +420224382730, e-mail: cadkovae@af.czu.cz, fax: +420234381801

Abstract

This study investigates the sorption of tebuconazole in contrasting soil types (Rendzic Leptosol, Haplic Arenosol and Haplic Cambisol). Tebuconazole was used both as an analytical grade chemical and as a commercial product. Sorption experiments were performed using the batch equilibrium approach. The sorption of analytical grade tebuconazole in soils was lower compared to the commercial product with the exception of the Cambisol. The highest sorption was observed for the Cambisol regardless of the tebuconazole form. In the experiment with the commercial formula containing tebuconazole, a higher sorption was observed for the Leptosol compared to the Arenosol; the experiment with analytical grade tebuconazole showed an opposite trend. The differences in sorption of the commercially available and analytical grade tebuconazole can be attributed to the additives present in the commercial product. Based on the obtained results, tebuconazole sorption is mainly influenced by soil organic matter and the mineralogical composition, but fungicide composition plays and important role as well. Additives present in the commercial products support fungicide sorption in soils. These findings confirm that tebuconazole is adsorbed in soils with high contents of organic matter and clay minerals and its application to such soils should not lead to risks associated with fungicide leaching.

Keywords: Additives, Azole, Fungicide, Sorption

Introduction

Tebuconazole [(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-pentan-3-ol] belongs to the chemical class of triazoles. It is widely used as a fungicide controlling soil born and foliar fungal pathogens. In general, the consumption of triazoles and diazoles has slowly increased in the Czech Republic as well as in the world (FAOSTAT, 2011);

therefore, studies dealing with environmental behavior of these fungicides are needed to better understand and evaluate the risks associated with their use. The frequent application of tebuconazole could result into its accumulation in the agricultural soils and consequently adversely affect soil ecosystems (Komárek et al., 2010). In general, adsorption is the main process influencing pesticides accumulation and movement in soil profiles (Arias-Estévez et al., 2008) and is mainly affected by physico-chemical properties of the pesticide (Table 1) and soil properties (Gevao et al., 2000). According to Kamrin (1997), tebuconazole is considered to have low solubility in water and slightly mobile in the environment SSLRC (Soil Survey and Land Research Centre, Cranfield University, UK). Its relatively high log K_{ow} value indicates that tebuconazole is rather sorbed to soils and sediments and should not be subjected to extensive leaching (Chamberlain et al., 1996). Additionally, tebuconazole degradation and its possible accumulation in soils is significantly influenced by the content of soil organic matter (SOM) (Berenzen et al., 2005), which is in accordance with its relatively high value of the distribution coefficient K_{oc} (describing pesticide distribution between organic carbon and the liquid phase). Pesticide sorption is also influenced by other soil components especially clays (Singh, 2005) and Fe-, Mn-oxyhydroxides (Sukop and Cogger, 1992). All these factors indicate that tebuconazole should be preferentially sorbed to soils or sediments other than leached through soil profiles (FAO, 1994). Nevertheless, tebuconazole has been detected in small surface water streams situated in agricultural environments (Berenzen et al., 2005) probably as a consequence of runoff. Therefore, tebuconazole behavior in soils should be investigated and monitored.

To our best knowledge there is only limited information concerning tebuconazole sorption onto contrasting soil types. Additionally, commercial pesticide formulations usually contain additives to increase their effectiveness and their influence on tebuconazole sorption is also investigated in this study. In our case, N,N-dimethyldecanamide is present in the commercial formulation prevents the recrystallization of the fungicide. Additives in commercial formulations can influence the water-soil partition and increase fungicide sorption (Pose-Juan et al., 2010). Based on these hypotheses, this study is also focused on the different behavior of tebuconazole when used as an analytical grade chemical and as a commercial product.

Materials and Methods

Soils and Chemicals

Soils with different physico-chemical characteristics (Rendzic Leptosol, Haplic Arenosol and Haplic Cambisol) were chosen for this study (Table 2). Detailed information concerning the methods of soil characterization can be found in Vaněk et al. (2010). Analytical grade tebuconazole was supplied by the Institute of Industrial Organic Chemistry (Poland, Warsaw) with a purity of 99.9 ± 0.1%. The commercial product containing tebuconazole (Horizon 250 EW) was supplied by Bayer CropScience (Germany, Monheim). This commercial product is a mixture of organic additives and contains 25.9% of tebuconazole. All other chemicals were of analytical grade and supplied by Chromservis (Czech Republic, Prague).

Sorption Experiments

Sorption experiments were performed using the batch equilibrium approach. A stock solution of analytical grade tebuconazole at a concentration of 1 g L^{-1} was prepared in methanol and kept in cold (4 °C) and dark. Solutions of tebuconazole (at concentration of 10–45 mg L^{-1}) were prepared by adding the appropriate volume of stock solutions of tebuconazole to the background electrolyte, 0.01 M $CaCl_2$, which is recommended by the OECD Guideline 106 for sorption experiments (OECD, 2000). Similar concentration ranges were chosen by other authors (Herrero-Hernández et al., 2011). Methanol was subsequently removed using a rotary evaporator (Rotavapor R-215, Büchi, Switzerland, Flawil). Solutions of the commercial product Horizon 250 EW (10–45 mg L^{-1} of tebuconazole) were made up by adding the appropriate amount of the commercial product Horizon 250 EW to 0.01 M $CaCl_2$.

Volumes of 50 mL of the tebuconazole solutions at desired concentrations were added to 5 g of soil. Samples were shaken for 48 hours at 20±2 °C (GFL 3017 orbital shaker, Germany, Burgwedel), which was the time necessary to reach equilibrium (Table 3), centrifuged for 10 minutes at 7000 rpm (Hettich Universal 30 RF, Germany, Tuttlingen) and subsequently extracted by the organic solvent ethyl acetate by shaking the mixture for 1 hour. Appropriate volumes of ethyl acetate were removed and analyzed by GC/MS (Agilent Technologies 6890N/5975, USA, CA). The experiments were performed in dark and in triplicates.

The amount of tebuconazole sorbed onto the soil types was calculated from the difference between the initial tebuconazole concentration and the equilibrium concentration after the sorption.

The adsorption isotherms were fitted to the following equations:

(i) the Langmuir isotherm:

$$S = S_{\max} \times \frac{KC}{1+KC} \qquad (1)$$

(ii) the Freundlich isotherm:

$$S = K_f \times C^n \qquad (2)$$

where S is the sorbed concentration (μmol g^{-1}), C is the aqueous equilibrium concentration (μmol L^{-1}), S_{max} is the maximum sorbed concentration (μmol g^{-1}), K is the Langmuir binding-strength coefficient (L μmol^{-1}); constants K_f (L g^{-1}) and n (dimensionless) characterize the adsorption capacity and intensity. The isotherms were created using non-linear fitting based on the sum of least squares (Bolster and Hornberger, 2007). Goodness of fit (E) was calculated according to Bolster and Hornberger (2007):

$$E = 1 - \frac{\sum_{i=1}^{N} w_i \left(S_i - f_i\right)^2}{\sum_{i=1}^{N} \left(w_i S_i - S_{wavg}\right)^2} \qquad (3)$$

where E is a goodness of fit, N is the number of observations, w_i is the i*th* weighting factor, S_i is the i*th* measured value of the dependent variable, f_i is the i*th* model-predicted value of the dependent variable and S_{wavg} is the weighted mean of the measured values. A model efficiency of 1 indicates a perfect fit to the data.

Results and Discussion

Sorption Experiments Using Analytical Grade Tebuconazole

All sorption isotherms describing the sorption of analytical grade tebuconazole better fit the Langmuir isotherm (Figure 1). Values of the Freundlich (K_f, n) and Langmuir parameters (S_{max}, K) and the goodness of fit (E) are shown in Table 4. The highest S_{max} value (sorption) was found for the Cambisol (6.32 μmol g^{-1}; E=0.99), which are supported by the higher Freundlich parameters. Analytical grade tebuconazole was sorbed more efficiently on the Arenosol (1.45 μmol g^{-1}; E=0.96) compared to the Leptosol (0.86 μmol g^{-1}; E=0.95), even though the Arenosol is lower in SOM. However, the Arenosol contained a higher amount of hydrophobic neutral organic matter that could support tebuconazole sorption. The higher sorption ability of the Arenosol was probably also caused by the higher clay content and the clay composition. The Arenosol and Cambisol contain clinochlore belonging to the chlorite group that was found to be able to adsorb aliphatic compounds and hence could contribute to the relatively high Arenosol sorption ability. Even though SOM is considered to be the most important factor influencing pesticide sorption, our data show that other factors are important as well. Singh (2005) also reported lower sorption in a soil that was higher in SOM in comparison to other soils and concluded that the type/nature of the clays can play an important role in sorption of azole-based fungicides. It is not obvious, which of the mentioned effects is the most important factor influencing tebuconazole sorption. However the data confirm the importance of soil characteristics in prediction of tebuconazole behavior in soils and the possibility to reach surface waters and groundwater.

Sorption Experiments Using Commercially-Available Tebuconazole

Figure 1 summarizes results of tebuconazole sorption (analytical grade and commercial product) in the studied soils. Values of the Freundlich (K_f, n) and Langmuir parameters (S_{max}, K) and goodness of fit (E) are shown in Table 5. The highest S_{max} value (2.76 µmol g^{-1}; E=0.97) was found for the Cambisol followed by the Leptosol (2.16 µmol g^{-1}; E=0.98) and the Arenosol (1.94 µmol g^{-1}; E=0.81). These results are again supported by the Freundlich parameters (Table 5). The highest sorption ability of the Cambisol can be attributed to the highest amount of SOM as well as the highest amount of clay particles and oxalate-extractable Al and Mn, corresponding roughly to amorphous and poorly crystalline oxyhydroxides. The Cambisol also contained the highest amount of hydrophobic neutral organic matter that interacts more strongly with hydrophobic organic pollutants (Table 2) and thus support tebuconazole sorption. Even though the SOM contents in the Leptosol and Arenosol are different, both studied soils exhibited a similar trend of tebuconazole sorption, i.e., similar sorption curves (Figure 1). However, a higher S_{max} value was found for the Leptosol. A relatively high sorption on the Arenosol (even though it exhibited the lowest SOM content) occurred probably due to the higher clay content (compared to the Leptosol). As mentioned earlier the Arenosol and Cambisol contain clinochlore that can contribute to the relatively high Arenosol sorption ability. Additives (e.g., N,N-dimethyldecanamide) present in commercial products could thus be easily adsorbed onto chlorite and subsequently support tebuconazole sorption through cooperative adsorption (Zhang et al., 2007). The similar ability of those two soils to sorb tebuconazole can be also explained by the similar amounts of oxalate-extractable Fe and Mn in both soils and points out the importance of these soil components in tebuconazole sorption (Sukop and Cogger, 1992). One reason explaining the Arenosol`s lowest S_{max} value could be the higher content of fulvic acids that are known to compete with the fungicide for the adsorption sites.

Our results correspond to findings reported by other authors. Čadková et al. (2011) and Rodríguez-Cruz et al. (2006) found that content and quality of soil organic matter play an important role during the sorption of azole-based fungicides. Kaiser et al. (2001) reported that hydrophobic neutral organic matter can significantly support the interactions with hydrophobic organic pollutants, including tebuconazole. It is in a good agreement with the sorption trend found in the Cambisol that contains the highest amount of these soil components. However, other authors have shown a significant role of clay minerals and other soil components (e.g., Fe-, Mn-oxyhydroxides) in fungicide sorption (Sukop and Cogger, 1992; Koutsopoulou et al., 2010). Again this study confirms these findings. Koutsopoulou et al. (2010) found that chlorites are able to adsorb aliphatic compounds and hence contribute higher sorption ability of soils as well as Fe-, Mn-oxyhydroxides content, corresponding to the results obtained for the Arenosol and the Leptosol.

Sorption of tebuconazole as a commercial product showed a higher S_{max} values in the case of the Arenosol and Leptosol. The differences found in sorption of tebuconazole as a commercial product and as an analytical grade chemical can be attributed to the additives (e.g., N,N-dimethyldecanamide) present in the commercial product that support fungicide effectiveness, simplifies its application and prevents the crystallization of the fungicides. Tebuconazole belongs due to its physico-chemical properties to lipophilic compounds and exhibit thus a high affinity for SOM. The additives in commercial products are mostly of organic origin, which can support tebuconazole sorption by co-adsorption of tebuconazole and the additives (Čadková et al., 2011). A similar trend was observed by Pose-Juan et al. (2010) and Rodríguez-Cruz et al. (2006) who found that surfactants or other additives can increase fungicides sorption in soils.

As mentioned earlier, tebuconazole is considered as slightly mobile and should not be found in deeper soil profiles. Tebuconazole is mainly accumulated in the top soil layer (0–10 cm) and higher retention was recorded in soils amended with organic materials. Nevertheless, tebuconazole was identified in deeper horizons, especially after increased application doses and also with high organic material amendments (Herrero-Hernández et al., 2011). It implies that excessive organic matter addition can possibly enhance tebuconazole mobility, possibly due the competition of dissolved organic matter for the sorption sites, and highlights the importance of SOM quality (i.e., content of humic and fulvic compounds). Other azole-based fungicides such as penconazole, hexaconazole, propiconazole and triadimefon were also more mobile in soils higher in SOM content. Despite the fact that penconazole and hexaconazole moved through the soil profile down to 10–15 cm, the majority was retained in the top 5 cm. Propiconazole was found in soil profiles down to 20 cm (Singh, 2005). On the other hand, soils low in SOM can exhibit a similar trend. Even though 97% of propiconazole remained in the upper soil layer, 4.4% of applied propiconazole was found in the leachate of a sandy loam soil (Kim et al., 2002). The mobility of propiconazole as well as tebuconazole is also influenced by the vegetation cover, which reduces the mobility of these fungicides (Gardner and Branham, 2001; Dousset et al., 2010). Additionally, the persistence of azole fungicide is associated with SOM content, clay content, vegetation cover or soil moisture (Strickland et al., 2004; Dousset et al., 2010; Herrero-Hernández et al., 2011). On the other hand Strickland et al. (2004) observed a high rate of tebuconazole dissipation in a soil low in organic matter. The authors explain this trend by a relatively high temperature and soil moisture that contributed to low tebuconazole sorption and thus higher bioavailability. Enhanced fungicide dissipation can be as well a result of repeated applications (Potter et al., 2005).

Based on our results and results obtained by other authors, it can be assumed that SOM content and quality (in terms of hydrophobic neutral organic matter content and content of humic, fulvic and hydrophilic acids) is one of the main factors influencing azole based fungicides (tebuconazole) sorption, mobility and persistence in the environment, but the contribution of clay minerals and Fe, Mn-oxyhydroxides cannot be ignored. There are also other important factors involved in this process such as the rate of applied fungicide, vegetation cover, temperature, soil moisture and other environmental conditions.

Conclusion

This study confirmed that tebuconazole sorption and subsequently its mobility and persistence in soils are mainly controlled by SOM and mineralogical contents. Tebuconazole application should be hence performed according to the physico-chemical properties of the soils. Climatic characteristics of the regions can also influence tebuconazole behavior in soils and tebuconazole applications should consider environmental conditions in the area. Additionally, soil amendments, such as organic matter application or clay amendments can improve soil quality and reduce the risks associated with the leaching of tebuconazole. The upcoming research should focus on other factors, such as the interactions of tebuconazole with other organic and inorganic fungicides and their influence on sorption in soils.

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Table 1 Physico-chemical properties of tebuconazole according to IUPAC (2011) and FAO (1994)

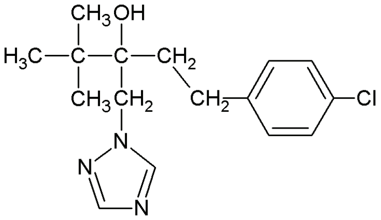
Chemical name	(*RS*)-1-*p*-chlorophenyl)-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol
CAS Number	107534-96-3
Chemical class	Triazoles
Formula	
Molecular mass	307.8
Water solubility (g L^{-1})	0.036 (at 20 °C)
Melting point (^{o}C)	105
log K$_{ow}$	3.7 (at 20 °C)
K$_{oc}$ (L kg $^{-1}$)	803–1251
Degradation in soils (d)	55.8–365

Table 2 Physico-chemical characteristics of the studied soils (Vaněk et al., 2010)

	Leptosol	**Arenosol**	**Cambisol**
Particle size distribution (%)			
Clay	0.4	1.3	3.2
Silt	30.6	6.4	32.6
Sand	69.0	92.3	64.2
pH $_{\text{water}}$	7.4	6.7	5.6
pH $_{\text{KCl}}$	7.0	6.4	4.7
pH $_{\text{ZPC}}$	7.7	6.5	5.1
CEC (cmol kg^{-1})	28.3	10.1	31.5
TOC (%)	3.36	1.16	3.78
TIC (g kg^{-1})	3.10	b.d.l.	b.d.l.
Oxalate extractable (g kg^{-1})			
Fe	2.23	1.91	1.63
Al	1.55	1.14	2.55
Mn	0.30	0.10	0.69
Minerals identified			
	quartz calcite albite orthoclase illite kaolinite	quartz muscovite albite orthoclase illite clinochlore clinopyroxene amphibole	quartz muscovite albite orthoclase illite clinochlore
Humic substances (g kg^{-1} d.m.)			
Humic acids	3.18	2.23	4.39
Fulvic acids	0.16	0.32	0.66
Hydrophilic acids	0.66	0.60	2.10
Hydrophobic neutral organic matter	0.29	0.62	0.83

b.d.l.: below detection limit

Table 3 Kinetic experiments with contrasting soil types; C_s-sorbed tebuconazole concentration at equilibrium; symbols represent: C_{init}-initial concentration, SD-standard deviation (n=2)

		Tebuconazole in commercial form				**Analytical grade tebuconazole**			
Soil type	**Time (h)**	**C_s (μmol g^{-1})** C_{init}=10 mg L^{-1}	**SD**	**C_s (μmol g^{-1})** C_{init}=45 mg L^{-1}	**SD**	**C_s (μmol g^{-1})** C_{init}=10 mg L^{-1}	**SD**	**C_s (μmol g^{-1})** C_{init}=45 mg L^{-1}	**SD**
Cambisol	24	0.21	0.02	1.30	0.01	0.21	0.01	0.95	0.01
	48	0.24	0.01	1.34	0.02	0.24	0.01	0.96	0.01
	72	0.24	0.01	1.34	0.01	0.24	0.03	0.97	0.01
Arenosol	24	0.14	0.03	0.92	0.02	0.08	0.02	0.48	0.01
	48	0.15	0.01	0.93	0.05	0.09	0.01	0.49	0.01
	72	0.16	0.01	0.94	0.04	0.11	0.01	0.49	0.02
Leptosol	24	0.18	0.01	0.90	0.03	0.09	0.01	0.54	0.02
	48	0.20	0.02	0.95	0.04	0.11	0.02	0.56	0.01
	72	0.21	0.01	0.95	0.04	0.12	0.01	0.57	0.01

Table 4 Fitting parameters of the Freundlich and Langmuir adsorption isotherms using analytical grade tebuconazole

Soil type	**Freundlich isotherm parameters**			**Langmuir isotherm parameters**		
	K_f (L g^{-1})	**n**	**E**	**K (L μmol^{-1})**	**S_{max} (μmol g^{-1})**	**E**
Cambisol	0.05	0.91	0.99	0.01	6.32	0.99
Arenosol	0.03	0.75	0.95	0.01	1.45	0.96
Leptosol	0.08	0.53	0.94	0.05	0.86	0.95

Table 5 Fitting parameters of the Freundlich and Langmuir adsorption isotherms using tebuconazole in commercial form

Soil type	Freundlich isotherm parameters			Langmuir isotherm parameters		
	K_f (L g^{-1})	n	E	K (L μmol^{-1})	S_{max} (μmol g^{-1})	E
Cambisol	0.04	0.84	0.96	0.01	2.76	0.97
Arenosol	0.01	0.96	0.97	0.002	1.94	0.81
Leptosol	0.02	0.68	0.97	0.003	2.16	0.98

Figure 1. Tebuconazole sorption onto contrasting soil types; C_s-sorbed tebuconazole concentration at equilibrium; C_{eq}-final solute tebuconazole concentration at equilibrium; symbols represent: CP-commercial formulation, AGT-analytical grade tebuconazole. Values shown are means ± SD (n=3).

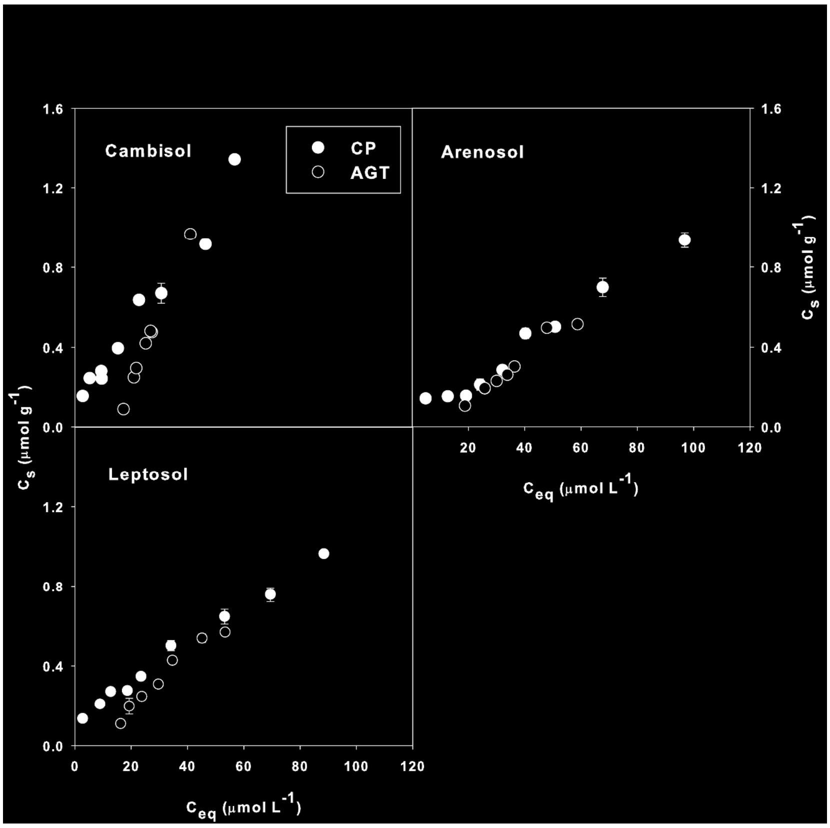


Figure 1. Tebuconazole sorption onto contrasting soil types; Cs-sorbed tebuconazole concentration at equilibrium; Ceq-final solute tebuconazole concentration at equilibrium; symbols represent: CP-commercial formulation, AGT-analytical grade tebuconazole. Values shown are means ± SD (n=3).
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The influence of copper on tebuconazole sorption onto soils, humic substances and ferrihydrite

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Corresponding Author:	Eva Čadková CZECH REPUBLIC
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Corresponding Author's Institution:	
Corresponding Author's Secondary Institution:	
First Author:	Eva Čadková
First Author Secondary Information:	
Order of Authors:	Eva Čadková
	Michael Komárek
	Regina Kaliszová
	Jiřina Száková
	Aleš Vaněk
	François Bordas
	Jean-Claude Bollinger
Order of Authors Secondary Information:	
Abstract:	The aim of this study is to investigate how the presence of Cu influences tebuconazole (Teb) sorption onto contrasting soil types and two important constituents of the soil sorption complex: hydrated Fe oxide and humic substances. Tebuconazole was used in commercial form and as an analytical-grade chemical at different Teb/Cu molar ratios (1:4, 1:1, 4:1 and Teb alone). Increased Cu concentrations had a positive effect on tebuconazole sorption onto most soils and humic substances, probably as a result of Cu−Teb tertiary complexes on the soil surfaces. The sorption of analytical-grade tebuconazole onto all matrices was lower, but the addition of Cu supported again tebuconazole sorption. The differences in tebuconazole sorption can be attributed to the additives present in the commercial product. This work proved the importance of soil characteristics and composition of the commercially available pesticides together with the presence of Cu on the behavior of tebuconazole in soils.
Suggested Reviewers:	Manuel Arias-Estévez University of Vigo mastevez@uvigo.es -expert on pesticide sorption and degradation in soils
	Ida Lykke Fabricius Technical University of Denmark ilf@env.dtu.dk -expert on pesticide sorption onto soils and separate soil components
	Neera Singh

	Indian Agricultural Research Institute drneerasingh@yahoo.com -expert on behavior of azole based fungicides in soil profiles
Opposed Reviewers:	

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The influence of copper on tebuconazole sorption onto soils, humic substances and ferrihydrite

Eva Čadková[a]*[1], Michael Komárek[b], Regina Kaliszová[a2] Jiřina Száková[a], Aleš Vaněk[a], François Bordas[c], Jean-Claude Bollinger[c]

[a] Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic (kaliszova@af.czu.cz, szakova@af.czu.cz, vaneka@af.czu.cz)
[b] Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic (komarek@fzp.czu.cz)
[c] Groupement de Recherche Eau, Sol, Environnement (GRESE), Université de Limoges, 123 avenue Albert Thomas, 87060 Limoges, France (francois.bordas@unilim.fr, jean-claude.bollinger@unilim.fr)
[1] Present address: Czech Geological Survey, Geologická 6, 152 00 Prague 5, Czech Republic (eva.cadkova@geology.cz)
[2] Present address: ALS Czech Republic, Na Harfě 336/9, 190 00 Prague 9, Czech Republic, (regina.kaliszova@alsglobal.cz)

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* Corresponding author: Czech Geological Survey, Geologická 6, 152 00 Prague 5, Czech Republic, tel. +420251085332, fax. +420251818748, e-mail: eva.cadkova@geology.cz

ABSTRACT

The aim of this study is to investigate how the presence of Cu influences tebuconazole (Teb) sorption onto contrasting soil types and two important constituents of the soil sorption complex: hydrated Fe oxide and humic substances. Tebuconazole was used in commercial form and as an analytical-grade chemical at different Teb/Cu molar ratios (1:4, 1:1, 4:1 and Teb alone). Increased Cu concentrations had a positive effect on tebuconazole sorption onto most soils and humic substances, probably as a result of Cu−Teb tertiary complexes on the soil surfaces. The sorption of analytical-grade tebuconazole onto all matrices was lower, but the addition of Cu supported again tebuconazole sorption. The differences in tebuconazole sorption can be attributed to the additives present in the commercial product. This work proved the importance of soil characteristics and composition of the commercially available pesticides together with the presence of Cu on the behavior of tebuconazole in soils.

KEYWORDS: copper; ferrihydrite; fungicide; soil organic matter; sorption; azole

Introduction

Until the 1930s, inorganic S- and Cu-based fungicides had been the most common chemicals used to protect crops against fungal diseases. The long-term application of these compounds led to their accumulation in soils and other environmental compartments (Komárek et al. 2010). Since the 1930s, a number of synthetic organic fungicides have been developed (Washington 2002). Tebuconazole [(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)-pentan-3-ol], belonging to the chemical class of triazoles, is a frequently used fungicide for the protection of crops (e.g., cereals, mustard, oilseed rape, hop, grape vine, stone fruits, peanuts, vegetables such as pepper) and wooden materials. The use of tebuconazole and other azole-based fungicides has rapidly increased in the last decade in several countries (e.g., Czech Republic, Italy, United Kingdom, Lithuania, Finland, Bangladesh, Japan, Uruguay) (FAOSTAT 2011); therefore, studies dealing with the environmental behavior of this fungicide are needed to better understand and evaluate the risks associated with its use. Long-term applications of tebuconazole can result in its accumulation in soil profiles, which can consequently affect soil ecosystems (Bending et al. 2007). Despite the fact that Cu-based fungicides have been commonly used in agriculture (e.g., viticulture) together with other organic fungicides, including tebuconazole, information about their interactions is lacking.

Tebuconazole behavior in soil profiles, including its degradation, is controlled by sorption processes. Its sorption is mainly influenced by soil organic matter (SOM) content (FAO 1994; Čadková et al. 2012), which is in accordance with its relatively high value of the distribution coefficient K_{oc} (describing pesticide distribution between organic carbon and the liquid phase). According to its K_{oc} value, tebuconazole is considered to be slightly mobile in soil profiles with a strong affinity to soil organic carbon (FAO 1994). However, other authors showed that pesticide sorption can be significantly affected by other soil components, especially clays and Fe-, or Mn-oxyhydroxides (Sukop and Cogger 1992).

The ability of Cu to form complexes with tebuconazole can influence the extent of their sorption and mobility in soil profiles (Jaklová Dytrtová et al. 2011). The relatively long tebuconazole half-life in soils (56–365 days) (IUPAC, 2012) allows the formation of such Cu-tebuconazole complexes in the soil system. Arias et al. (2006) observed that the presence of Cu in soils resulted into a higher sorption of penconazole, another triazole fungicide, probably due to the formation of Cu-penconazole complexes, which exhibited a higher affinity for soil colloids. However, these processes have not been investigated in detail.

The interactions of Cu with organic pesticides can also influence their toxicity. Jacobson et al. (2005) found that increased Cu concentrations influenced the fate and transport of diuron in soil profile. The changes were probably caused by different composition of microbiological populations, which could lead to subsequent changes in activities involved in their degradation. On the other hand, diuron was found to prevent Cu-induced decreases of chlorophyll contents in some plants (Teisseire et al. 1999). Therefore, further studies concerning changes in tebuconazole toxicity in the presence of Cu and *vice versa* should be performed.

Most of the previous studies were performed using technical grade tebuconazole, despite the fact that the pure form is not used in agriculture and commercially available products contain many other additives that support fungicide effectiveness and simplify its application. It is needed to point out that the additives in pesticide formulations can significantly influence the sorption mechanisms and behavior in soils (Čadková et al. 2012).

To our best knowledge, there is no study describing Cu and tebuconazole interactions and their influence on sorption and behavior in contrasting soils, including ferrihydrite and humic substances (HS). Comparison of tebuconazole sorption in commercial form and analytical-grade and their interactions with Cu has not been studied either.

The aim of this study is to evaluate whether the presence of Cu can influence the sorption of tebuconazole (Teb) onto different soils and their important constituents, such as Fe oxyhydroxide (ferrihydrite) and soil organic matter, represented here by humic substances. The experiments were conducted at different Teb : Cu molar ratios (1 : 4, 1 : 1, 4 : 1 and Teb alone) to find out to what extent Cu affects tebuconazole sorption through the formation of Cu-tebuconazole complexes. Another objective is to study whether the fungicide formulation (commercial vs. analytical-grade) can influence the interactions between Cu and tebuconazole and their sorption in chosen matrices.

Materials and methods

Soils and chemicals

Soils with different physico-chemical characteristics (Rendzic Leptosol, Haplic Arenosol and Haplic Cambisol) were chosen for this study (**Table 1**). Detailed information concerning the methods of soil characterization can be found in Vaněk et al. (2010). Two-line ferrihydrite was synthesized according to the procedure described by Schwertmann and Cornell (2000). The so-called “humic substances” or “humic acids”, used as a model of SOM, were purchased from Sigma-Aldrich (USA). According to Malcolm and MacCarthy (1986), humic acids purchased from Sigma-Aldrich consists of C 63.15, H 5.60, O 34.98, N 0.80, S 4.58, $P < 0.05$ (% by weight ash-free and moisture-free basis), ash 22.56% with atomic ratios C/N 92 and H/C 1.06. The determination of hydrophobic neutral organic matter, humic, fulvic and hydrophilic acids contents in the soil samples was performed according to the method developed by van Zomeren and Comans (2007).

Analytical-grade tebuconazole was supplied by the Institute of Industrial Organic Chemistry (Poland) with a purity of 99.9±0.1%. The main physico-chemical properties of tebuconazole are shown in **Table 2**. The commercial fungicidal product containing tebuconazole Horizon 250 EW was supplied by Bayer CropScience (Germany). Analytical pure $Cu(NO_3)_2.3H_2O$, $CaCl_2$ and KNO_3 were purchased from Lach-Ner (Neratovice, Czech Republic). All other chemicals were of analytical grade and supplied by Chromservis (Czech Republic).

Tebuconazole p*K*a determination

In order to evaluate the dissociation behavior of tebuconazole, its acid dissociation constant (pK_a) was determined by potentiometric titration in a 20% (v/v) acetonitrile/water solvent mixture, according to a slight modification of the method described by Fikri et al. (2011). Tebuconazole (0.01 g) was dissolved in 20% acetonitrile (100 mL; at a fixed 0.1 M ionic strength using KNO_3). Nitric acid (0.1 M) was added (in the same molar quantity as tebuconazole) to 20 mL of the tebuconazole solution and subsequently titrated by 0.01 M KOH in a thermostated cell (25 ºC) under nitrogen atmosphere (Linde, 5.0). The exact concentration of the KOH solution was determined by titration with nitric acid (0.1 M; Normadose Prolabo - VWR, France). All reagents and acetonitrile were of analytical grade (Fluka, Switzerland). All the solutions were prepared from ultra-pure carbonate-free water and the titration started after pH equilibrium was reached in the cell. The titration was performed using the automatic titrator Metrohm 716 DMS Titrino coupled to a Metrohm 727 Ti Stand, a Metrohm 722 stirrer and equipped with a combined pH electrode (Metrohm, pH 0−14/0−80 °C; KCl 3 mol L^{-1}) (Switzerland). The set parameters are the dynamic mode, a 1 mV min^{-1} signal drift and a 5 mL min^{-1} maximal flow-rate. The combined glass electrode was calibrated with pH 7.00 and 4.00 aqueous buffers and then with a 0.05 M potassium hydrogenophthalate solution in 20% acetonitrile solvent (Longhi et al. 1986). All procedures were performed in triplicates. The titration curves were modeled using Protofit (version 2.1) software (Turner and Fein 2006) to calculate the pK_a values using the Davies activity coefficient model. According to Fikri et al. (2011), this calculated value can be considered as numerically equal to the value in pure water, within the experimental uncertainties (± 0.05).

Preparation of solutions containing tebuconazole and Cu

Analytical-grade tebuconazole stock solution at concentration 1 g L^{-1} was prepared in methanol and kept in a cold and dark place. Aqueous solutions of tebuconazole (either as analytical-grade or as commercial formulation) at concentrations of 10−35 mg L^{-1} in experiments with soils and at concentrations 50−175 mg L^{-1} in experiments with ferrihydrite and humic substances were prepared by adding the appropriate volume of tebuconazole methanolic stock solution to 0.01 M $CaCl_2$. This background electrolyte was chosen due to its wide use in other studies performing similar experiments (e.g., Clausen and Fabricius 2001) and is recommended by the OECD Guideline 106 for sorption experiments (OECD 2000). Methanol was subsequently removed using a rotary evaporator (Rotavapor R-215, Büchi, Switzerland). The $CaCl_2$ aqueous medium improves centrifugation and minimizes cation exchange (OECD 2000). Copper solutions were prepared by dissolving the appropriate amounts of $Cu(NO_3)_2.3H_2O$ in 0.01 M $CaCl_2$ in order to obtain various Teb : Cu molar ratios (1 : 4, 1 : 1, 4 : 1).

Sorption experiments

Sorption experiments were performed using the batch equilibrium approach. In experiments involving soils, a volume of tebuconazole solutions (40 mL) and $Cu(NO_3)_2.3H_2O$ solutions (10 mL) at desired concentrations were added to soil (5g). Previous batch kinetic experiments showed that 48 hours was long enough to reach the equilibrium (data not shown). Therefore, samples were shaken for 48 hours (GFL 3017 orbital shaker, Germany) and centrifuged for 10 min at 5752 × *g* (Hettich Universal 30 RF, Germany). Changes of pH were monitored during the experiment. A volume of 10 mL was removed and Cu concentrations were analyzed using ICP-OES (Vista Pro, Varian, Australia). The rest of the supernatant was extracted by the organic solvent ethyl acetate through shaking the mixture for 1 h. An appropriate volume of ethyl acetate was removed to vials and tebuconazole content was analyzed using GC/MS (Agilent Technologies 6890N/5975, CA, USA). The calibration curve was constructed using seven concentrations and the correlation coefficient was always at least 0.995. Calibration solutions for external calibration were prepared in the same way as all samples (without the matrices). The calibrations and result evaluation were made according to general US EPA 8000C method (2003). Measurement uncertainty was determined based on the validation.

Ferrihydrite (0.39 g L^{-1}) (Clausen and Fabricius 2001) or humic substances (1.00 g L^{-1}) (Iglesias et al. 2009) were equilibrated in 0.01 M $CaCl_2$ for 24 hours. Ferrihydrite suspension was adjusted to pH 5.5 with HCl and $Ca(OH)_2$. The pH value of the humic substances suspensions was not adjusted because of its high pH-buffering capacity and reached 3.3 at equilibrium. A volume of tebuconazole solution (10 mL) and $Cu(NO_3)_2.3H_2O$ solution (10 mL) at the desired concentrations were added to suspensions of ferrihydrite/humic substances (30 mL) and shaken for 48 h. The subsequent analytical procedures were performed as mentioned earlier. All experiments were performed in dark and in triplicates.

The amount of tebuconazole and Cu sorbed onto the soils, ferrihydrite, or humic substances was calculated as follows:

$$S = \frac{(C_i - C)}{m} \times V \qquad (1)$$

where S is the sorbed concentration (μmol g^{-1}), C_i is the initial concentration in solution (μmol L^{-1}), C is the aqueous equilibrium concentration (μmol L^{-1}), V is the volume of the liquid phase (L) and m is the mass of soil/ferrihydrite/humic substances in the suspension (g).

The adsorption isotherms were constructed using the Freundlich isotherm equation:

$$S = K_f \times C^n \qquad (2)$$

where Freundlich constants K_f (L g^{-1}) and n, a measure of the nonlinearity involved (dimensionless), characterize the adsorption capacity and intensity. The Freundlich isotherms were created using the non-linear (sum of least squares) model using a solver Excel file developed by Bolster and Hornberger (2007). Goodness of fit (E) was calculated according to Bolster and Hornberger (2007):

$$E = 1 - \frac{\sum_{i=1}^{N} w_i (S_i - f_i)^2}{\sum_{i=1}^{N} (w_i S_i - S_{wavg})^2} \qquad (3)$$

where E is a goodness of fit, N is the number of observations, w_i is the i*th* weighting factor, S_i is the i*th* measured value of the dependent variable, f_i is the i*th* model-predicted value of the dependent variable and S_{wavg} is the weighted mean of the measured values. A model efficiency of 1 indicates a perfect fit to the data.

Results and discussion

Tebuconazole p*K*a determination

Dissociation of compounds is often associated with the pH value of the given system (e.g., soil) and the determination of tebuconazole pK_a could possibly help to explain its behavior under different pH conditions. Furthermore, the dissociation of tebuconazole is an important precursor of its potential complexation with Cu. Until now, there has been limited information about the dissociation constant of tebuconazole. Tebuconazole is considered as a weak base, which can only be completely protonated in non-aqueous systems in the presence of very strong acids (IUPAC 2012), although Chimuka et al. (2009) cited the tebuconazole`s pK_a value to be 3.39. In our experiments, we determined the pK_a value for the equilibrium between the protonated and the free molecular tebuconazole:

$Teb{\cdot}H^+ \leftrightarrows Teb + H^+$ pK_a = 5.03 (at a fixed 0.1 M ionic strength using KNO_3 and 25 °C).

Theoretically, H^+ dissociation from protonated tebuconazole should occur when soil pH is higher than tebuconazole pK_a value. The pH values of soils used in our experiments varied from 5.6 to 7.4. All values are in a close range of tebuconazole pK_a. Accordingly, based on previous results, it can be considered that soil pH does not affect tebuconazole dissociation and consequently its sorption (Čadková et al. 2012; Čadková et al. in press).

As mentioned earlier tebuconazole p*K*a is an important precursor of its potential complexation with Cu. Based on a suggestion by Evans et al. (2007), the formation constants of the $CuTeb^{2+}$, $Cu(Teb)_2^{2+}$, $Cu(Teb)_3^{2+}$ and $Cu(Teb)_4^{2+}$ complexes (log β) could be estimated as approximately equal to $\log \beta_1 = 4.2$, $\log \beta_2 = 7.8$, $\log \beta_3 = 10.7$, $\log \beta_4 = 12.8$. The speciation of Teb : Cu complexes was modeled using MINEQL+ (Schecher and McAvoy 2001) within the soil solution (in the pH range of 4–10) for various Teb : Cu ratios (in 0.01 M $CaCl_2$ as background electrolyte, with chloro-complexes included). It was found that mainly $CuTeb^{2+}$ and $Cu(Teb)_2^{2+}$ are possibly formed in solution, which is in a good agreement with our earlier study (Jaklová Dytrtová et al. 2011). The following maximal values of formed complexes were obtained for the pH range 5–7: 60% of $CuTeb^{2+}$ and 8% of $Cu(Teb)_2^{2+}$ for a Teb : Cu ratio of 1 : 4; 35% $CuTeb^{2+}$ and 10% $Cu(Teb)_2^{2+}$ for 1 : 1; and 12% $CuTeb^{2+}$ and 8% $Cu(Teb)_2^{2+}$ for 4 : 1.

Sorption experiments using analytical-grade tebuconazole

Figures 1 and **2** describe the sorption of analytical-grade tebuconazole on the studied soils, humic substances and ferrihydrite. All sorption isotherms describing the sorption of analytical grade tebuconazole fitted the Freundlich isotherm. Values of the Freundlich parameters (K_f, n) and goodness of fit (E) are shown in **Table 3**.

As mentioned earlier, the content and quality of SOM play an important role during the sorption of azole-based fungicides (Čadková et al. 2012; Čadková et al. in press) as well as the presence of Cu in soils that possibly supports sorption of azole-based fungicides through the formation of Cu-azole complexes (Arias et al. 2006). Concerning these findings in general, humic substances (representing soil organic matter) and the Cambisol should show the highest ability to adsorb tebuconazole and also higher Cu concentrations should support tebuconazole sorption to all matrices. Our data show that in experiments without Cu addition, the highest tebuconazole sorption in soils was observed for the Cambisol followed by the Arenosol and the Leptosol, even though the order of decreasing SOM content is: the Cambisol > the Leptosol > the Arenosol. It implies that the quality of SOM in terms of humic, fulvic and hydrophilic acids contents and content of hydrophobic neutral organic matter can significantly influence tebuconazole sorption. Many authors (Sparks 2003; Hiller et al. 2009) reported the higher ability of humic acids to sorb organic compounds compared to fulvic acids. Nevertheless, chemical structures and sorption properties of the humic and fulvic acids vary depending mainly on their origin, age, and environmental parameters such as vegetation or temperature. Differences of more than one order of magnitude in sorption coefficients of a given compound measured in humic and fulvic acids from different sources of various origins were found (Niederer et al. 2007). The Cambisol contains the highest amount of humic acids, which confirmed the theory that these substances have higher ability to sorb organic compounds. Although the Leptosol contained higher amount of humic acids (compared to the

Arenosol; **Table 1**), it showed lower tebuconazole sorption ability. It was probably caused by its origin and other factors mentioned earlier. On the contrary, the Cambisol and the Arenosol both contain a high amount of fulvic acid as well. They should exhibit lower binding affinity for nonpolar organic chemicals than humic acids. In general, fulvic acids are more polar, depict a higher O/C ratio and a higher amount of oxygen-containing functional group (Sparks 2003). These factors could negatively influence interactions between fulvic acids and nonpolar organic chemicals (Hiller et al. 2009). Even though tebuconazole is a nonpolar compound and the Cambisol and the Arenosol contain high amounts of fulvic acids, they exhibited high tebuconazole sorption ability. Again, it was probably caused by the nature and origin of the fulvic acids.

Singh (2005) also reported a lower sorption in a soil that was higher in SOM content in comparison to other soils and concluded that the type/nature of the clay minerals and other soil components present could play an important role in sorption of azole-based fungicides. The different clay composition of the studied soils could be one of the reasons explaining the lower tebuconazole sorption in the Leptosol. The Arenosol and the Cambisol contain clinochlore (as opposed to the Leptosol), which could support tebuconazole sorption (Koutsopoulou et al. 2010). Both soils also contain muscovite, which was found to be able to sorb other azole-based fungicide (Rodríguez-Cruz et al. 2008). The other possible reason why the Leptosol showed a lower tebuconazole sorption is that it also contains a high amount of calcite that did not show ability to sorb this fungicide (Čadková et al. 2012).

Regardless of the Teb : Cu ratio, the highest sorption ability was found for humic substances and ferrihydrite, followed by the Cambisol, the Leptosol and the Arenosol. The highest tebuconazole sorption ability on materials rich in organic matter (humic substances representing SOM and Cambisol, containing the highest amount of SOM) was observed for the samples without Cu followed by the Teb : Cu ratios 1 : 4 ≥ 1 : 1 > 4 : 1. The highest tebuconazole sorption for samples that did not contain Cu was probably caused by the competition of Cu and tebuconazole for the adsorption sites of humic acids (Xu et al. 2005). The authors observed a competitive behavior between Cu and an organic herbicide (propisochlor), while the sorption of Cu on humic acids was not remarkably affected. Also, while analytical-grade tebuconazole does not contain any additives that support its sorption as in the case of the commercial formulation (see section 3.3), it is probably most easily adsorbed on SOM than on other soil components, especially due to its high affinity for organic matter (FAO 1994; Čadková et al. 2012; Čadková et al. in press). This assumption is supported by the experiment with ferrihydrite, where the amount of sorbed tebuconazole from solutions without Cu was negligible. On the contrary, higher tebuconazole sorption from solutions containing Cu was observed for ferrihydrite, indicating the positive impact of the complexation on sorption. This finding could explain as well tebuconazole sorption on the Arenosol containing the lowest amount of SOM and a relatively high oxalate-extractable Fe concentration, indicating a relatively high content of amorphous or poorly crystalline Fe oxides. Again, increased Cu concentrations most probably supported tebuconazole sorption due to the formation of Cu-tebuconazole complexes. It seems that at the lower concentrations of Cu (Teb : Cu 4 : 1 and 1 : 1) there are not sufficient amounts of these formed complexes (see our data in section 3.1) and the extent of tebuconazole sorption is lower compared to samples without Cu addition. A similar trend was found for the Leptosol, even though the Leptosol contains a relatively high SOM content. The highest sorption was observed for the Teb : Cu ratio of 1 : 4, followed by the sample without Cu addition ≈ 1 : 1 ≈ 4 : 1. The differences between the amounts of sorbed tebuconazole at different Cu concentrations are not as evident as in the experiment with other matrixes with the exception of the Teb : Cu ratio of 1 : 4. The isotherms show clearly that the presence of Cu promotes tebuconazole sorption due to the neutral or slightly negative charge of solid surfaces at the experimental pH values. Therefore, the positively charged complexes can be sorbed more efficiently to the matrices.

According to the obtained K_f values (**Table 3**), there is an evidence of Cu supporting tebuconazole sorption (affinity) to all matrices. The Teb : Cu ratio with the highest Cu addition (1 : 4) exhibited the highest K_f values in all matrices (in comparison to other Teb : Cu ratios) with the exception for ferrihydrite, where the highest K_f value was found for Teb : Cu ratio 1 : 1. The Cambisol and the Leptosol exhibited the following order of K_f values: Teb : Cu ratios 1 : 4 > 1 : 1 > 4 : 1 > samples without Cu and Teb : Cu ratios 1 : 4 > 4 : 1 > 1 : 1 > samples without Cu addition, respectively. It is thus clear that Cu addition increased tebuconazole's affinity for the sorbents most possibly through the formation of Cu-tebuconazole complexes. A similar trend was observed for ferrihydrite. The trend was slightly different in the Arenosol soil: Teb : Cu ratios 1 : 4 > samples without Cu addition > 1 : 1 > 4 : 1. As mentioned above, the relatively high tebuconazole affinity to soil samples without Cu addition, was probably caused by the lowest SOM content and the high content of amorphous or poorly crystalline Fe oxides (e.g., ferrihydrite). The highest K_f value was observed for humic substances followed by ferrihydrite, the Cambisol, the Leptosol and the Arenosol. It again implies that SOM plays an important role in tebuconazole sorption. Also the shape of the curves confirms this assumption. The curve showing tebuconazole sorption onto humic substances is more like the shape of curves depicting tebuconazole sorption in contrasting soil types than to ferrihydrite. The main interactions between soil and tebuconazole are then probably mainly influenced by SOM. Nevertheless, the role of other constituents, such as Fe- and/or Mn-oxides and clay minerals cannot be omitted and it is difficult to specify the contribution of each specific constituent.

Sorption experiments using commercially-available tebuconazole

Figures 1 and **2** summarize the results of the sorption of tebuconazole from the commercial formulation on the studied soils, humic substances and ferrihydrite. Values of the Freundlich parameters (K_f, n) and goodness of fit (E) are shown in **Table 3**. The highest sorption was found for the humic substances and ferrihydrite, followed by the Cambisol, the Arenosol and the Leptosol, regardless the Teb : Cu ratio. The highest sorption ability of the Cambisol can be explained by the highest amount of SOM and possibly by the highest portion of the clay fraction and oxalate-extractable Al and Mn. The Cambisol also contained the highest amount of hydrophobic neutral organic matter that interacts more strongly with hydrophobic organic pollutants (**Table 1**) (Kaiser et al. 2001). The Arenosol exhibited a higher tebuconazole sorption even though its SOM content is lower than in the Leptosol. This is probably caused by the higher clay content (compared to the Leptosol). As mentioned above, the Arenosol and the Cambisol contain clinochlore belonging to the chlorite group that was found to be able to adsorb aliphatic compounds (Koutsopoulou et al. 2010) and hence could contribute to the high sorption ability of the Arenosol. Furthermore, the commercial product contains additives (up to 60% w/w N,N-dimethyldecanamide) that could thus be easily adsorbed onto chlorite and subsequently support tebuconazole sorption through cooperative adsorption (Zhang et al. 2007). The high ability of the Arenosol to sorb tebuconazole can be also explained by the relatively high amounts of oxalate-extractable Fe, Al and Mn, which confirms the importance of these soil components in tebuconazole sorption (Sukop and Cogger 1992).

In experiments without Cu addition, the highest tebuconazole sorption was observed for the Cambisol, followed by the Leptosol and the Arenosol. This order could be explained by the amount of SOM content, because higher soil organic matter content increases tebuconazole sorption in soils. Concerning different Cu concentrations in sorption experiments with contrasting soil types, tebuconazole sorption increased in the following order of different Teb : Cu ratios 1 : 4 > 1 : 1 > 4 : 1 > without Cu addition, with the only exception for the Leptosol. The same trend was observed for the humic substances. Again, the highest Cu content led to the highest sorption of tebuconazole. As mentioned earlier, higher Cu concentrations in soils resulted in higher sorption of azole-based fungicides through formation of Cu-azole complexes, which exhibited a higher affinity for soil components (Arias et al. 2006). Interestingly, the Leptosol showed a different trend of tebuconazole sorption, according to the Teb : Cu ratio: without Cu addition > 1 : 4 > 1 : 1 ≈ 4 : 1. A similar trend was observed for ferrihydrite: tebuconazole sorption was significantly higher in samples containing no Cu. It could be concluded that during tebuconazole sorption on ferrihydrite, it competes for adsorption sites with other components, e.g., Cu and hence shows the highest tebuconazole sorption ability in samples that do not contain any Cu. Another explanation could be that the sorption of the Teb-Cu complexes is not favored on the ferrihydrite surface. On the other hand, in samples containing different Cu concentrations, tebuconazole sorption followed the trend (for the ratio Teb : Cu): 1 : 4 > 1 : 1 ≈ 4 : 1. Again there was sufficient time during the batch experiment to form Cu-tebuconazole complexes that would support tebuconazole sorption. These assumptions could explain the different trend in tebuconazole sorption in the Leptosol as well. The Leptosol contains the highest amount of oxalate-extractable Fe, corresponding roughly to amorphous and poorly crystalline oxyhydroxides, and tebuconazole is thus slightly more adsorbed from solutions without Cu addition. As in previous experiments using analytical grade tebuconazole, the presence of Cu enhanced tebuconazole sorption (except for the Leptosol and ferrihydrite) and the positively charged Teb-Cu complexes were sorbed more easily to the negatively charged solid surfaces.

According to the obtained K_f values (**Table 3**), tebuconazole exhibited the lowest affinity to all soil types without Cu addition. Again tebuconazole showed the highest affinity to the Cambisol and the Arenosol when the Teb : Cu ratio was 1 : 4, followed by the ratios 1 : 1 and 4 : 1. The highest tebuconazole affinity to Leptosol was found for the Teb : Cu ratio 1 : 1 > 4 : 1 > 1 : 4. Again, Cu addition increased tebuconazole affinity to all soil types and the opposite trend was observed for ferrihydrite, where tebuconazole exhibited the highest affinity to samples without Cu addition, followed by Teb : Cu ratios 4 : 1 > 1 : 4 > 1 : 1. As mentioned earlier, the presence of Cu does not enhance tebuconazole sorption onto the Fe oxide and the Teb-Cu complexes are not preferentially sorbed. The highest K_f value was observed for humic substances followed by ferrihydrite, the Cambisol, the Arenosol and the Leptosol. Again, SOM as well as mineral and clay composition seem to play an important role in tebuconazole sorption. Additionally, tebuconazole sorption in the Arenosol and the Leptosol seemed to occur in two steps. First, tebuconazole was less intensively adsorbed on both soil types at low concentrations (0.2– 0.3 µmol g^{-1}), while in the second step, tebuconazole sorption and affinity increased (up to 0.53– 1.24 µmol g^{-1} for the Leptosol and 1.16– 2.68 µmol g^{-1} for the Arenosol). It was probably caused by the increasing concentration of additives in the commercial formula (see below).

The sorption patterns of commercially available tebuconazole differ from the experiment using tebuconazole as a pure chemical. These differences can be attributed to the additives that are mainly of organic molecules (e.g., N,N-dimethyldecanamide). These additives support fungicide effectiveness, simplify its application and prevent tebuconazole recrystallization. They could be easily adsorbed on the matrices and subsequently support tebuconazole sorption by the so-called cooperative adsorption (Zhang et al. 2007). In a study that compared the sorption ability of natural minerals and minerals modified with organic surfactants, the adsorption efficiency of natural minerals was much lower compared to the modified minerals. Sorption efficiency of azoles onto such modified clay minerals can be thus increased up to 133-times due to the high organic carbon content of organic surfactants (Rodríguez-Cruz et al. 2008).

Conclusion

It could be concluded that one of the main factor influencing tebuconazole sorption soils is the content and quality of SOM although the contribution of clay minerals and Fe-, Mn-oxyhydroxides (especially ferrihydrite) cannot be neglected. Nevertheless it is difficult to determine the specific role of each component in tebuconazole sorption. Our results show the importance of soil characteristics and the presence of metallic elements (e.g., Cu from other fungicides) for predicting tebuconazole behavior in soils. Influence of additives that are present in commercially available products cannot be ignored either. Our data confirm the possible positive effect of these substances on tebuconazole sorption. As mentioned earlier the interactions of Cu with organic pesticides can also influence their toxicity in the soil environment. Therefore, studies concerning interactions of tebuconazole and Cu and influence on their toxicity should be examined in detail.

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Fig. 1 Tebuconazole sorption onto contrasting soil types; C_s-sorbed tebuconazole concentration at equilibrium; C_{eq}-final solute tebuconazole concentration at equilibrium; symbols represent: CF-commercial formulation, AG-analytical-grade tebuconazole. Values shown are means ± SD (n=3)

Fig. 2 Tebuconazole sorption onto humic substances and ferrihydrite; C_s-sorbed tebuconazole concentration at equilibrium; C_{eq}-final solute tebuconazole concentration at equilibrium; symbols represent: CF-commercial formulation, AG-analytical-grade tebuconazole. Values shown are means ± SD (n=3)

Figure 1

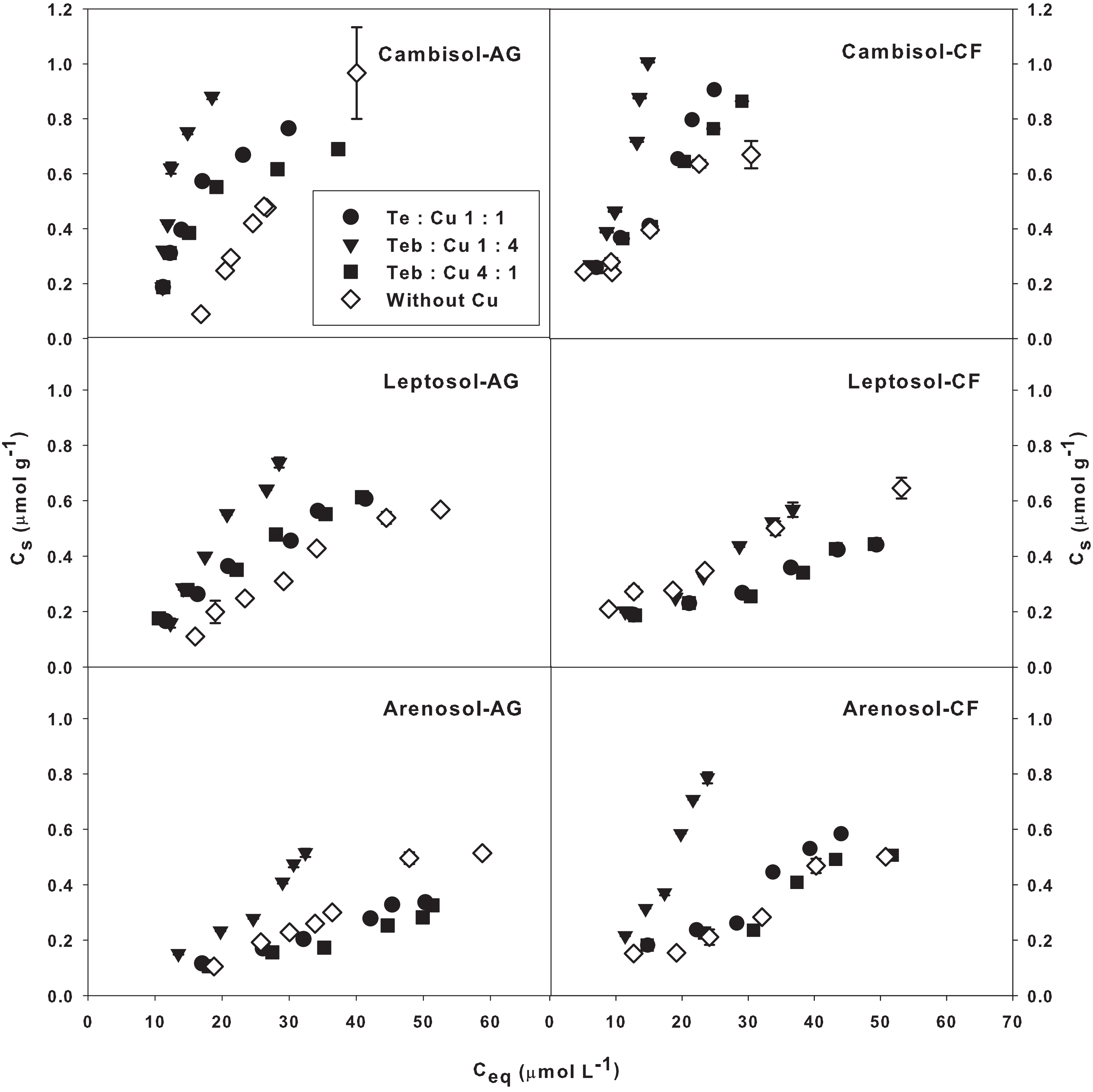
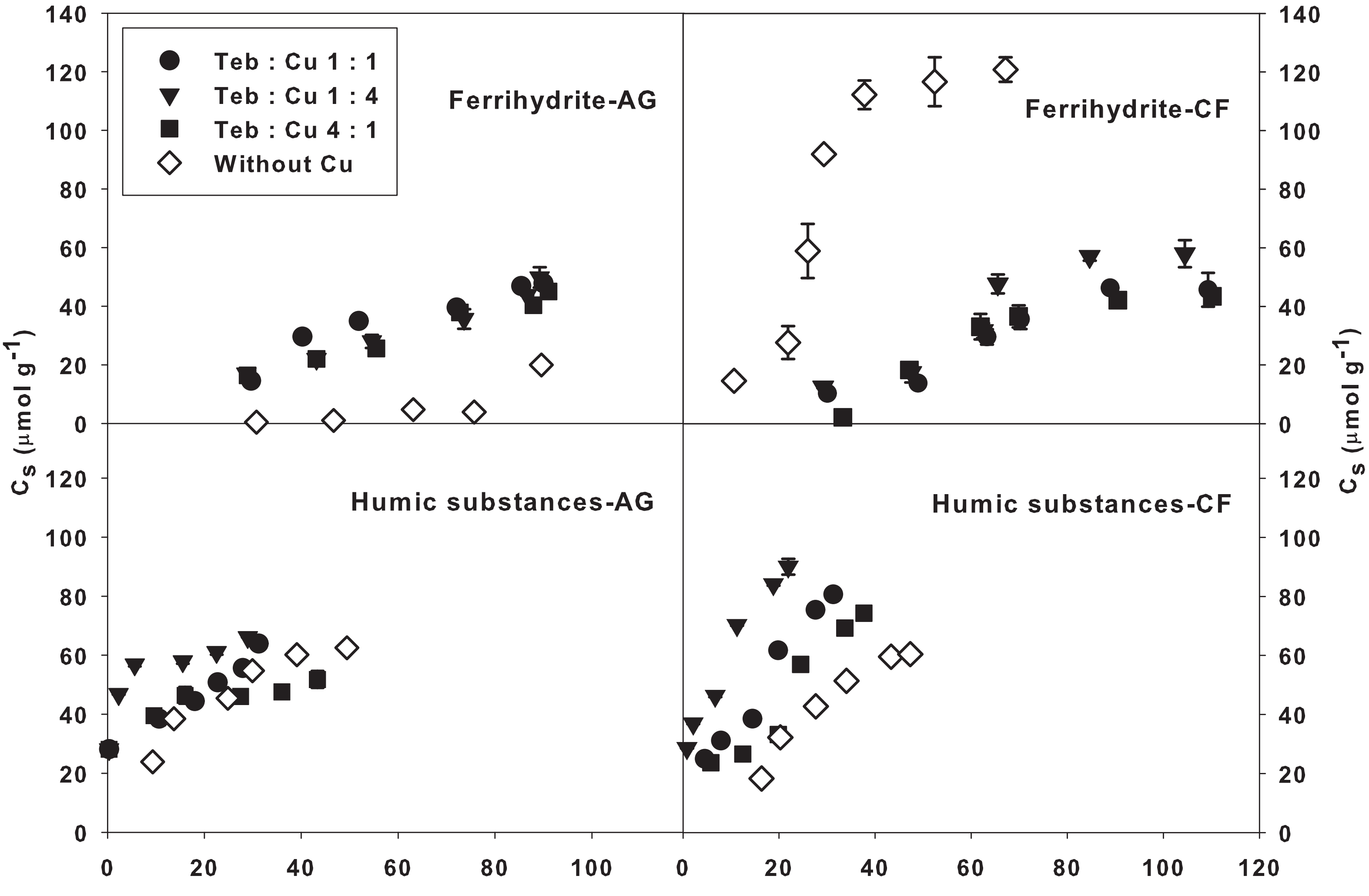

Cambisol-AG
Cambisol-CF
Leptosol-AG
Leptosol-CF
Arenosol-AG
Arenosol-CF
● Te : Cu 1 : 1
▼ Teb : Cu 1 : 4
■ Teb : Cu 4 : 1
◇ Without Cu
C_s (μmol g^{-1})
C_{eq} (μmol L^{-1})
0.0
0.2
0.4
0.6
0.8
1.0
1.2
0
10
20
30
40
50
60
70

Figure 2



Teb : Cu 1 : 1
Teb : Cu 1 : 4
Teb : Cu 4 : 1
Without Cu
Ferrihydrite-AG
Ferrihydrite-CF
Humic substances-AG
Humic substances-CF
C_S (μmol g^-1)
C_eq (μmol L^-1)
0
20
40
60
80
100
120
140

Table 1

Table 1 Physico-chemical characteristics of the studied soils (Vaněk et al. 2010)

	Leptosol	**Arenosol**	**Cambisol**
Particle size distribution (%)			
Clay	0.4 ± 0.09	1.3 ± 0.3	3.2 ± 0.2
Silt	30.6 ± 1.5	6.4 ± 0.4	32.6 ± 1.0
Sand	69.0 ± 2.1	92.3 ± 0.8	64.2 ± 0.6
pH $_{water}$	7.4 ± 0.2	6.7 ± 0.1	5.6 ± 0.2
pH $_{KCl}$	7.0 ± 0.2	6.4 ± 0.2	4.7 ± 0.1
pH $_{ZPC}$	7.7 ± 0.7	6.5 ± 0.3	5.1 ± 0.5
CEC [a] (cmol kg^{-1})	28.3 ± 2.3	10.1 ± 1.7	31.5 ± 2.8
TOC [b] (%)	3.36 ± 0.45	1.16 ± 0.21	3.78 ± 0.57
TIC [c] (g kg^{-1})	3.10 ± 0.29	b.d.l.	b.d.l.
Oxalate extractable (g kg^{-1})			
Fe	2.23 ± 0.25	1.91 ± 0.33	1.63 ± 0.25
Al	1.55 ± 0.30	1.14 ± 0.19	2.55 ± 0.24
Mn	0.30 ± 0.09	0.10 ± 0.07	0.69 ± 0.07
Minerals identified			
	quartz calcite albite orthoclase illite kaolinite	quartz muscovite albite orthoclase illite clinochlore clinopyroxene amphibole	quartz muscovite albite orthoclase illite clinochlore
Humic substances (% w/w)			
Humic acids	74.1 ± 4.3	59.2 ± 2.9	55.0 ± 1.0
Fulvic acids	3.7 ± 0.5	8.5 ± 1.4	8.3 ± 0.6
Hydrophilic acids	15.4 ± 0.9	15.9 ± 1.8	26.3 ± 2.1
Hydrophobic neutral organic matter	6.8 ± 0.5	16.4 ± 0.9	10.4 ± 0.6

b.d.l.: below detection limit

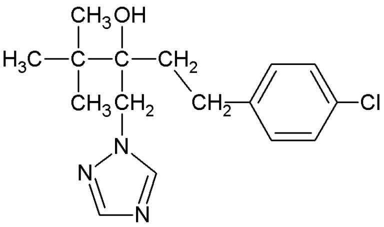
[a] Cation exchange capacity

[b] Total organic carbon content

[c] Total inorganic carbon content

Table 2

Table 2 Physico-chemical properties of tebuconazole (FAO 1994; IUPAC 2012)

Chemical name	(*RS*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol
CAS Number	107534-96-3
Chemical class	triazoles
Molecular mass	307.8
Formula	
pK_a [a]	5.03 (at 25 °C)
Water solubility (g L^{-1})	0.036 (at 20 °C)
Melting point (°C)	105
log K_{ow} [b]	3.7 (at 20 °C)
K_{oc} (L kg^{-1}) [c]	803–1251
Degradation in soils (d) [d]	55.8–365

[a] pK_a value determined in this study

[b] K_{ow} is the octanol/water partition coefficient

[c] Partition coefficient normalized to organic carbon content

[d] Aerobic soil half-life (days)

Table 3

Table 3 Fitting parameters of the Freundlich adsorption isotherms using analytical-grade tebuconazole and tebuconazole in commercial form

	Teb : Cu ratio	**Analytical-grade tebuconazole**			**Tebuconazole in commercial form**		
		K_f (L g^{-1})	**n**	**E**	**K_f (L g^{-1})**	**n**	**E**
Ferrihydrite	1 : 1	5.54	0.51	0.95	2.51	0.67	0.90
	1 : 4	3.09	0.63	0.94	3.05	0.69	0.87
	4 : 1	3.55	0.58	0.96	4.34	0.55	0.93
	without Cu	0.77	0.91	0.96	14.8	0.54	0.84
Humic substances	1 : 1	11.6	0.46	0.93	7.07	0.71	0.96
	1 : 4	23.6	0.30	0.76	8.62	0.72	0.98
	4 : 1	20.3	0.24	0.92	9.23	0.59	0.83
	without Cu	9.52	0.50	0.94	16.9	0.37	0.99
Cambisol	1 : 1	0.28	0.35	0.99	0.14	0.63	0.89
	1 : 4	0.33	0.49	0.88	0.22	0.66	0.86
	4 : 1	0.20	0.38	0.95	0.09	0.69	0.96
	without Cu	0.05	0.91	0.99	0.06	0.70	0.94
Arenosol	1 : 1	0.04	0.59	0.95	0.06	0.66	0.87
	1 : 4	0.07	0.61	0.96	0.15	0.65	0.88
	4 : 1	0.04	0.55	0.94	0.05	0.62	0.85
	without Cu	0.06	0.60	0.97	0.05	0.63	0.84
Leptosol	1 : 1	0.09	0.55	0.99	0.10	0.41	0.89
	1 : 4	0.14	0.58	0.99	0.08	0.60	0.91
	4 : 1	0.11	0.50	0.98	0.09	0.43	0.84
	without Cu	0.08	0.53	0.98	0.04	0.68	0.97

Supplementary Material

Journal of Solution Chemistry

pKa constant determination of two triazole pesticides: tebuconazole and penconazole

--Manuscript Draft--

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Corresponding Author:	jean-claude BOLLINGER Université de Limoges Limoges, FRANCE
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Université de Limoges
Corresponding Author's Secondary Institution:	
First Author:	Eva ČADKOVÁ, PhD student
First Author Secondary Information:	
Order of Authors:	Eva ČADKOVÁ, PhD student
	Michael KOMÁREK, PhD, hab.
	Jean DEBORD, MD, PhD, hab
	Loïc DELLA PUPPA, PhD student
	François BORDAS, PhD
	jean-claude BOLLINGER
Order of Authors Secondary Information:	
Suggested Reviewers:	Manuel ARIAS-ESTÉVEZ Prof., University of Vigo, Spain mastevez@uvigo.es a specialist of triazole fungicides and their environmental behavior
	Bogulaw BUSZEWSKI Prof., Faculty of Chemistry bbusz@chem.uni.torun.pl a specialist of HPLC analysis of triazole pesticides (using W/AN mixtures)
	Marti ROSÉS Prof., Universitat de Barcelona, Spain marti.roses@ub.edu a specialist of water + acetonitrile mixed solvents: potentiometric titrations, physico-chemical data, pKa determinations, HPLC, ...
	Ibrahim NARIN Prof., Faculty of Pharmacy narin@erciyes.edu.tr has recently published some papers about pKa determination (pharmaceutical compounds)

Université
de Limoges
GRESE
Groupement de Recherche
Eau Sol Environnement
IFR 145 GEIST

Professeur Jean-Claude BOLLINGER
Groupement de Recherches Eau - Sol - Environnement (GRESE)
Université de Limoges
123 Avenue Albert Thomas
87060 Limoges (France)
E-mail: jean-claude.bollinger@unilim.fr

to: The Editor
Journal of Solution Chemistry

Dear colleague,

As the senior (and corresponding) author, I have the pleasure to send to you the paper entitled:
“pKa constant determination of two triazole pesticides: tebuconazole and penconazole”
co-authored by
Eva ČADKOVÁ, Michael KOMÁREK, Jean DEBORD, Loïc DELLA PUPPA, François BORDAS and Jean-Claude BOLLINGER
submitted for publication in *Journal of Solution Chemistry*. We have prepared the manuscript, Tables and Figures according to the instructions for authors. We are convinced that you will find the manuscript acceptable in this form.

The topic fits the scope of the journal. To our knowledge, this is the first time that potentiometric acidity constants are determined for such triazole fungicides, and the discussion is completed with molecular modeling calculation about the protonation site. Such results are important for determining the behavior of these compounds in the soil environment where they are dispersed. All these results are new and they have not been submitted elsewhere.

At the time when the experimental work was conducted, EC was PhD student under the supervision of MK (PhD, hab), a Research Director at the Czech University of Life Sciences, Prague; JD (MD, PhD, hab) is a senior Research Associate at the University Hospital, Limoges; LDP is a PhD student, under the joint supervision of FB and MK; FB and JCB are Assistant Professor and Emeritus Professor, respectively, at the University of Limoges.

Following a query by MK, JCB designed the research project with the collaboration of JD and FB. During a short stay in Limoges, EC conducted the experiments with the participation of LDP, under the supervision of FB and JCB; data treatment was performed by EC, LDP and FB, and JD make the molecular modeling calculations. JCB wrote the first draft of the present paper, which has been completed and approved by all co-authors. The authors declare no competing financial interest.

We believe that the following reviewers would be suitable for our submission:

* Prof. Manuel ARIAS-ESTÉVEZ
Soil Science Laboratory
University of Vigo, Spain
E-mail: mastevez@uvigo.es

* Prof. Bogulaw BUSZEWSKI
Chair of Environmental Chemistry & Bioanalytics
Faculty of Chemistry
Nicolaus Copernicus University, Torun, Poland
E-mail: bbusz@chem.uni.torun.pl

* Prof. Marti ROSÉS
Dept. de Química Analítica
Universitat de Barcelona, Spain
E-mail: marti.roses@ub.edu

* Prof. Ibrahim NARIN
Department of Analytical Chemistry
Faculty of Pharmacy
Erciyes University, Kayseri, Turkiye
E-mail: narin@erciyes.edu.tr

Truly yours,

Prof. Jean-Claude BOLLINGER

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pK_a Constant Determination of Two Triazole Pesticides: Tebuconazole and Penconazole

Eva Čadková · Michael Komárek · Jean Debord · Loïc Della Puppa · François Bordas · Jean-Claude Bollinger

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Eva Čadková
Department of Agro-Environmental Chemistry and Plant Nutrition, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic; *present address*: Czech Geological Survey, Geologická 6, 152 00 Prague 5, Czech Republic

Michael Komárek
Department of Environmental Geosciences, Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Kamýcká 129, 165 21 Prague 6, Czech Republic

Jean Debord
Service de Pharmacologie-Toxicologie, Hôpital Dupuytren, 2 Avenue Martin Luther King, 87042 Limoges, France

Loïc Della Puppa · François Bordas · Jean-Claude Bollinger (✉)
Groupement de Recherche Eau, Sol, Environnement (GRESE), Université de Limoges, 123 avenue Albert Thomas, 87060 Limoges, France
e-mail: jean-claude.bollinger@unilim.fr

Abstract We determined the acidity constants of tebuconazole and penconazole, two fungicides from the group of 1,2,4-triazoles. Potentiometric titrations were performed in a 20% (v/v) acetonitrile/water mixture at 25 °C and at a fixed ionic strength (KNO_3, 0.1 mol·dm^{-3}). The pK_a values (representing thermodynamic constants) were determined to be 5.0 ± 0.1 and 5.2 ± 0.1 for tebuconazole and penconazole, respectively. These values could be used in pure water solutions to consider the protonated or deprotonated forms when studying the field behavior of these fungicides. Molecular modeling calculations allowed identifying the N4 atom as the protonation site.

Keywords Triazole pesticides · Potentiometric titrations · Protonation constant · Protonation site · Molecular modeling

1 Introduction

Agrochemicals include organic molecules, with various building blocks and functional groups. When they are dispersed in the field, their physical, chemical and ecotoxicological properties, mostly related to their structure, are of major importance for their environmental behavior. Among those, the acid/base properties are of special interest, because they determine the present chemical form: a neutral molecule or a charged ion (either a cation and/or an anion, depending on the molecular structure). Because the soil and its main components (clays, Fe- and Mn-oxyhydroxides, humic substances, etc.) exhibit different surface charges according to the environmental pH [1], the acidity constant K_a (or the pK_a) of the pesticides are crucial for determining their behavior in the soil environment.

Here we focused on two pesticides of the (benzo)triazole family for which some quantitative structure-properties relationships are already available [2]: tebuconazole and penconazole (Table 1, Fig. 1). They are known to behave as systemic fungicides, with both curative and protective actions, mainly applied to orchards and vineyards [3] where the soil pH is commonly within the range from 4 to 8. According to their structure (Fig. 1), both tebuconazole and penconazole can be expected to behave as weak bases, the N4 nitrogen atom in the 1, 2, 4-triazole ring being the most probable target for protonation (see section 3.1 below); unfortunately, their pK_a values are unknown (tebuconazole) or of dubious value (penconazole) [4]. The knowledge of their correct values is essential, in order to predict the nature of the species (neutral molecule or protonated one) present in the environment.

These triazole molecules are poorly water-soluble, and require water-miscible co-solvents in order to be solubilized. Acetonitrile is a very adequate cosolvent for use in RP-HPLC analysis of this class of compounds [5, 6] due to its aprotic and polar nature and because it is fully miscible with water. Therefore, a mixture of acetonitrile and water was chosen here for the potentiometric determination of the pK_a of the two fungicides.

2 Experimental

2.1 Reagents

Analytical-grade tebuconazole was supplied by the Institute of Industrial Organic Chemistry (Warsaw, Poland) with a purity of 99.9 ± 0.1 %; analytical standard penconazole was purchased from Fluka (Pestanal, 99.1 %). All reagents and acetonitrile (AN) were of analytical grade (Fluka), and dissolved in high purity de-ionized carbonate-free water (Milli-Q system: resistivity 18.2 MΩ·cm, TOC < 10 $\mu g \cdot dm^{-3}$).

2.2 Potentiometric Measurements

The pK_a values of tebuconazole and penconazole were determined by potentiometric titration in a 20% (v/v) acetonitrile/water solvent mixture, according to a slight modification of the method described by Fikri et al. [7]. Each triazole compound (0.01 g) was dissolved in 20% AN (100 cm^3; at a fixed 0.1 $mol \cdot dm^{-3}$ ionic strength using KNO_3), then sonicated (Bransonic model 20). Nitric acid (0.1 $mol \cdot dm^{-3}$) was added in equivalent amount to 20 cm^3 of the triazole solution and this mixture of strong acid and protonated weak base was subsequently titrated by 0.01 $mol \cdot dm^{-3}$ KOH (in the 20% AN solvent) in a thermostatted cell (25 ºC) under nitrogen atmosphere (Linde, 5.0). The exact concentration of the KOH solution was determined by titration with HNO_3 (0.1 $mol \cdot dm^{-3}$; Normadose Prolabo). The titration was performed (Fig. 2) using the automatic titrator Metrohm 716 DMS Titrino coupled to a Metrohm 727 Ti Stand, a Metrohm 722 stirrer and equipped with a glass/calomel pH electrode (Metrohm, pH 0–14/0–80 °C; KCl 3 $mol \cdot dm^{-3}$). The set parameters are the dynamic mode, a 1 $mV \cdot min^{-1}$ signal drift and a 5 $cm^3 \cdot min^{-1}$ maximal flow-rate. The titration started after pH equilibrium was reached in the cell. The combined glass electrode was calibrated with pH 7.00 and 4.00 aqueous buffers and then with a 0.05 $mol \cdot kg^{-1}$ potassium hydrogenophthalate solution in the 20% AN solvent ($pH_S = 4.58$ [8, 9]). All procedures were performed in triplicates.

The titration curves were fitted using the ProtoFit (version 2.1) software [10] to calculate the pK_a values and using the Davies activity coefficient corrections calculated from the data in the mixed solvent system [11]. According to Fikri et al. [7], such a calculated value can be considered as numerically equal to the value in pure water, within the experimental uncertainties (± 0.05).

2.3 Molecular Modeling

For each compound, the geometries of the two enantiomers were optimized by molecular mechanics (MM2 force field) with HyperChem [12] and further refined by the PM6 semi-empirical molecular orbital method with MOPAC [13, 14]. The gas phase formation enthalpies at 298 K were also computed from MOPAC.

3 Results and Discussion

3.1 Protonation Site

Due to the presence of a single chiral center (asymmetric carbon atom) in both molecular structures, each consists of two (*R* or *S*) enantiomers (Fig. 1), whose properties can be somewhat different; the studied samples were racemic mixtures, however.

According to our molecular theoretical quantum calculations of gas phase formation enthalpies (Table 3), the N4 protonation is favored by *ca.* 50–65 $\mathrm{kJ \cdot mol^{-1}}$, compared to N2 protonation, whatever the studied fungicide and its enantiomer form.[1]

3.2 Protonation Constants

The acidity constants, $\mathrm{p}K_a$, of the two triazole compounds were determined from potentiometric titrations in a 20% (v/v) AN solvent mixture at 25 °C. However it would be more useful to have information about the $\mathrm{p}K_a$ value in pure water. In order to estimate the effect of the 20% AN solvent mixture, we refer to our previous study on amino-2 pyridine derivatives [7]: it appeared that the values of aqueous $\mathrm{p}K_a$ would be *ca.* 0.02 unit higher than those determined in the 20% AN solvent, what is within the experimental uncertainties (± 0.05). Moreover, due to the introduction of activity coefficient corrections during data treatment, our calculated $\mathrm{p}K_a$ values can be considered as the thermodynamic ones. This is why we consider that our values are a good

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[1] We have also tested the new PM7 method in MOPAC 2012. This method, which is still under development, gave enthalpy values within 5.3% of the PM6 values. The relative stabilities of the protonated forms were unchanged.

estimate (better than ± 0.1 unit) of the aqueous pK_a of these two fungicides, *i.e.* for the equilibrium between the protonated and the free molecular triazole:

$$\mathrm{Teb{\cdot}H^+} \leftrightarrows \mathrm{Teb} + \mathrm{H^+} \qquad \mathrm{p}K_a = 5.0$$

$$\mathrm{Pen{\cdot}H^+} \leftrightarrows \mathrm{Pen} + \mathrm{H^+} \qquad \mathrm{p}K_a = 5.2$$

(± 0.1; at 25 °C and with a fixed 0.1 mol·dm^{-3} ionic strength using KNO_3)

Until now, there has been limited information about the dissociation constant of tebuconazole and penconazole (Tables 1 and 2). According to the IUPAC Pesticides Properties Database [4], tebuconazole is considered as a very weak base, without any numerical value; for penconazole, the low value given (1.51, see Table 1) is not in accordance with the structure of the compound, while it would correspond to a medium-strength acid. Few other data are cited in the literature (see Table 2); in all cases, they are lower than our experimental values, indicating a somewhat weaker basic behavior for the corresponding molecule, what is once more not coherent with its structure. Furthermore, the SPARC-calculated values [15] are too low to be realistic, and should be ignored.

4 Conclusion

The aqueous pK_a values, determined for the first time in this study, allow predicting the environmental behavior of either tebuconazole or penconazole according to the acid/base properties of the soil solution and of the soil sorption complex. If stability constants data become available, their possible complexation with metal cations present in the soil solution or added as pesticides (*e.g.*, Cu [17, 18]) can now be estimated, for a better knowledge of the speciation of these triazole fungicides.

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Table 1 Physico-chemical properties of the fungicides tebuconazole and penconazole, according to IUPAC Pesticides Properties Database [4]

	Tebuconazole	**Penconazole**
Chemical name	(*R,S*)-1-*p*-chlorophenyl-4,4-dimethyl-3-(1*H*-1,2,4-triazol-1-ylmethyl)pentan-3-ol	(*R,S*)-1-[2-(2,4-dichlorophenyl)pentyl]-1*H*-1,2,4-triazole
CAS Number	107534-96-3	66246-88-6
SMILES	Clc1ccc(cc1)CCC(O)(C(C)(C)C)Cn2ncnc2	Clc1ccc(c(Cl)c1)C(CCC)Cn2ncnc2
Chemical formula	$C_{16}H_{22}ClN_3O$	$C_{13}H_{15}Cl_2N_3$
Molecular mass	307.82	284.18
pK_a	not available 'very weak base'	1.51 (at 25 °C) 'very weak base'
Water solubility (g· dm^{-3} at 20 °C)	0.036	0.073
Melting point (ºC)	105	60.3
log K_{ow} [a]	3.7 (at pH 7 and 20 °C)	3.72 (at pH 7 and 20 °C)
K_{oc} (dm^3·kg^{-1}) [b]	769	2205
Degradation in soils (d) [c]	55.8	90.0
GUS leaching potential index [d]	2.00	1.51
BCF [e]	78	320

[a] K_{ow} is the octanol/water partition coefficient

[b] Partition coefficient normalized to organic carbon content, Freundlich model

[c] Aerobic soil half-life, field conditions (days)

[d] Groundwater Ubiquity Score (estimated)

[e] BioConcentration Factor

Table 2 pK_a values for the two fungicides

	Experimental results [a]	**Estimated value** [b]	**Literature data**
Tebuconazole	5.0 ± 0.1	1.56	3.39 [c]
Penconazole	5.2 ± 0.1	1.36	2.83 ± 0.12 [d]

[a] Potentiometric; $I = 0.1$ mol·dm^{-3}, 25 °C

[b] According to SPARC [15]

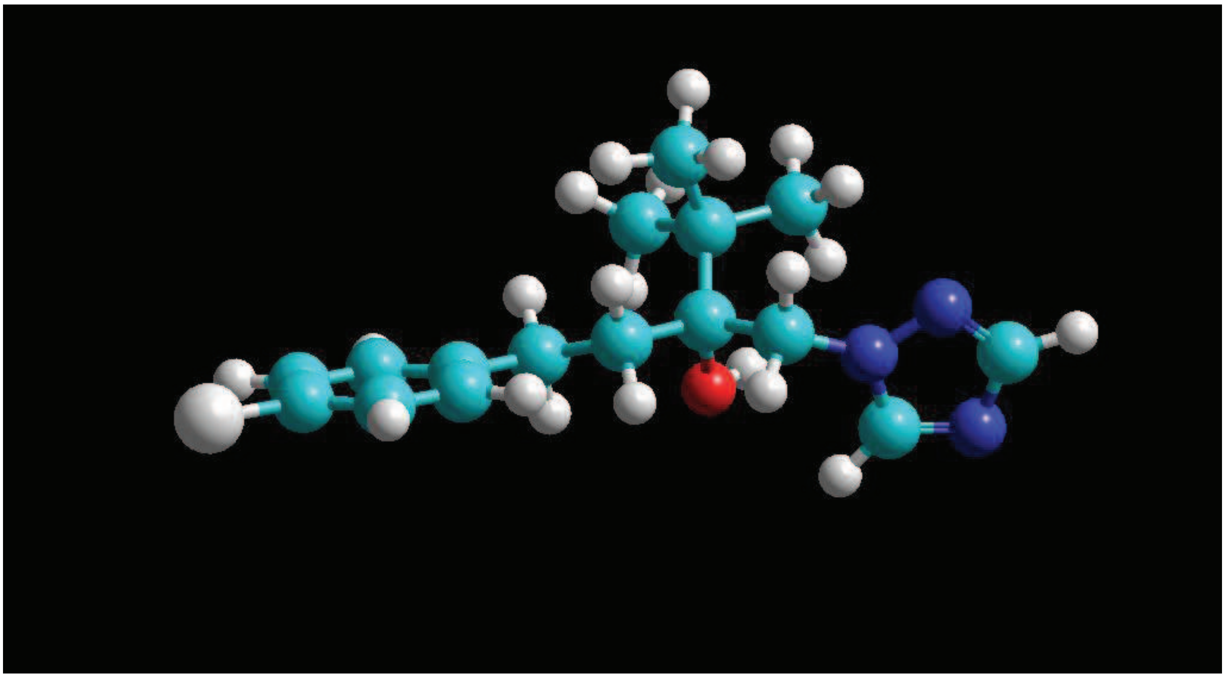
[c] Cited by Chimuka et al. [16]

[d] Cited by Arias et al. [17]

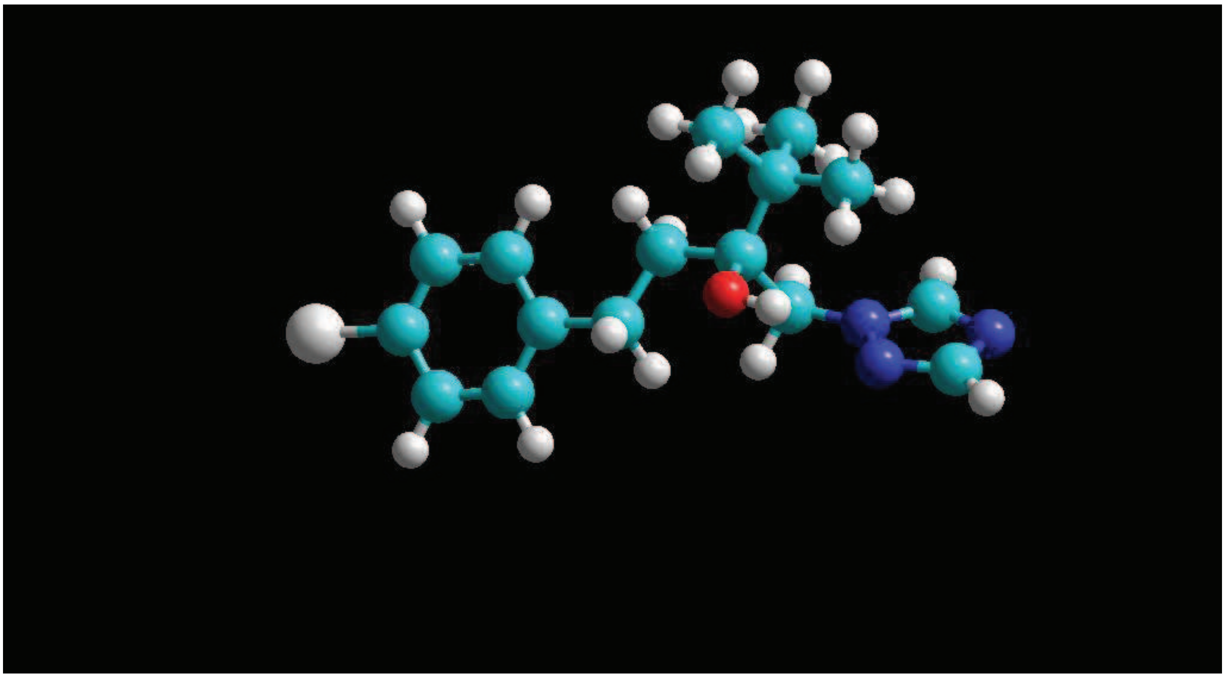
Table 3 Gas phase formation enthalpies (kJ·mol^{-1}) calculated with MOPAC/PM6

	Neutral molecule	**N2 protonated**	**N4 protonated**
Tebuconazole *R*	– 54.17	612.16	547.23
Tebuconazole *S*	– 46.90	602.16	551.14
Penconazole *R*	181.15	854.37	793.08
Penconazole *S*	183.27	850.82	795.17

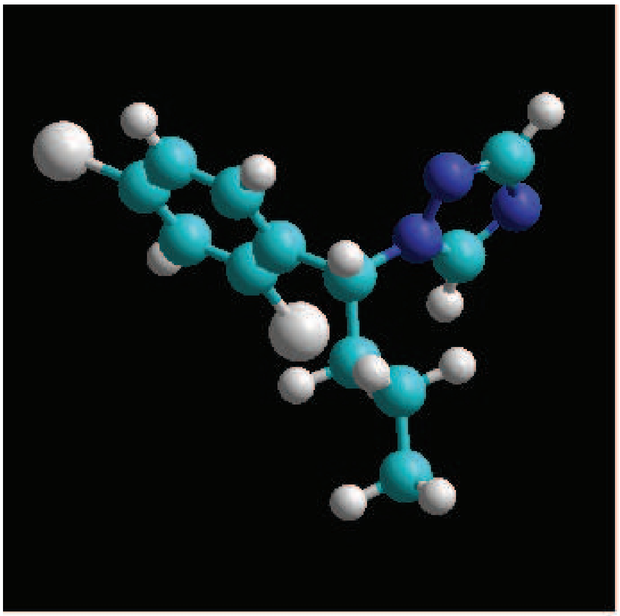
Fig. 1 Structures of Tebuconazole (a, b) and Penconazole (c, d) enantiomers (sticks and balls presentation).



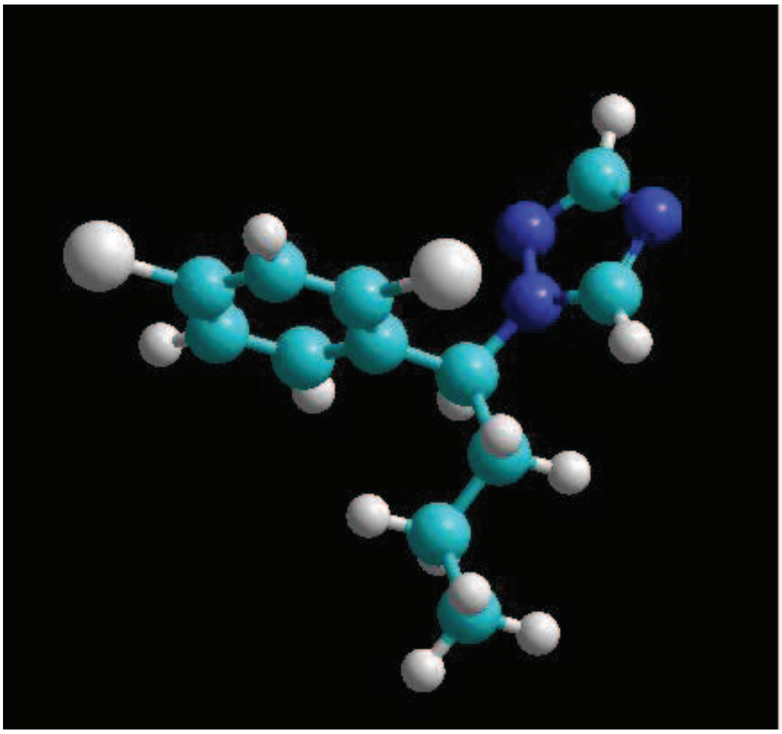
(a) Tebuconazole *R*



(b) Tebuconazole *S*



(c) Penconazole *R*



(d) Penconazole *S*

Fig. 2 Titration curves for Tebuconazole (×) and Penconazole (+) with 0.01 mol·dm^{-3} KOH in the 20% (v/v) AN/Water, 0.1 mol·dm^{-3} KNO_3 medium, after acidification with an equimolar amount of HNO_3.