Dissipation of herbicides in surface soils and subsurface sediment slurries and development of treatment methods

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ABSTRACT

Pesticides have been widely used in agriculture and also in non-agricultural areas, such as in parks and by railroads. Unfortunately, this has led to the contamination of many soils, subsoils, surface and ground waters. Pesticides tend to be more persistent in vadose and groundwater zones than in surface soils. When pesticide concentrations exceed the limit values for drinking water, the water needs to be cleaned. This motivated research on pesticide dissipation and development of methods for treatment of pesticide contaminated soils and groundwater.

Here, the dissipation of the herbicides atrazine and terbutryn was studied in five surface soils from the Boreal region, and the effects of oxygen, microbes and soil properties were examined. Herbicide dissipation was further studied in subsurface sediment slurries and in pilot-scale subsurface sediment columns. The subsurface sediments were from a groundwater area that was contaminated with atrazine, desethylatrazine (DEA), simazine and 2,6-dichlorobenzamide (BAM). The aim was to enhance the chemical or microbiological dissipation of herbicides with physical, chemical or microbiological treatment. The physical treatment comprised once or twice a day sonication for 0, 5, 10, 20 or 30 min (43 kHz, 320 W). Chemical approaches consisted of addition of surfactant methyl-β-cyclodextrin (0.5%), a mixture of zero-valent iron and organic matter (EHC®) (1% and 2%), or organic matter, i.e. peat (5%) or a compost-peat-sand (CPS) mixture (5% and 15%). The microbiological aspect included studying degradation by indigenous soil/sediment microbes compared with sterilized controls and bioaugmentation with *Pseudomonas* ADP.

Microbes enhanced the degradation of atrazine and terbutryn in surface soils, the half-lives being 57–181 d for atrazine and 70–291 d for terbutryn. Atrazine was also degraded chemically in four soils, with half-lives of 120–183 d. Oxygen did not have significant effect on the atrazine half-lives, while terbutryn was only degraded in aerobic soils. Atrazine and terbutryn dissipation was poorest in the soil that had the lowest amounts of organic matter, nitrogen, ammonium, nitrate and nitrite. Sonication did not decrease the atrazine concentrations, compared with the non-sonicated slurries and sterilized slurries, despite of indications of microbial and chemical degradation. Addition of surfactant did not stimulate atrazine degradation by indigenous sediment microbes, and *Pseudomonas* ADP degraded atrazine even without surfactant. EHC® enhanced atrazine dissipation chemically under aerobic conditions. EHC® in sediment columns cleaned atrazine-, DEA- and BAM-contaminated groundwater, compared with columns without EHC®, but the effect lasted only about 1 month. Peat and CPS enhanced the chemical dissipation of atrazine and simazine, regardless of the presence or absence of oxygen. Peat also enhanced hexazinone dissipation. Only trace amounts of atrazine, simazine and hexazinone could be extracted from peat and sediment, which indicates that the dissipation was caused by chemical degradation and/or unextractable bound residue formation.

In conclusion, the addition of peat, CPS and *Pseudomonas* ADP were the best approaches for reducing pesticide concentrations. EHC® could also be useful in remediation of small quantities of pesticides, preferably in the presence of oxygen. However, the usability of peat, CPS and EHC® in remediation should be further studied to avoid the possible adverse effects of organic matter additions to drinking water quality.

TIIVISTELMÄ

Torjunta-aineita on käytetty paljon maataloudessa sekä maatalouden ulkopuolella, kuten puistoissa ja rautateiden varsilla. Valitettavasti tämä on johtanut pintamaiden, syvempien kerroksien, vesistöjen ja pohjavesien pilaantumiseen. Torjunta-aineilla on taipumusta viipyä pidempään syvemmissä maakerroksissa ja pohjavesissä kuin pintamaissa. Kun juomavedelle asetettujen torjunta-ainepitoisuuksien raja-arvot ylittyvät, vesi on puhdistettava ennen kuin sitä voidaan käyttää. Tämä motivoi tutkimaan torjunta-aineiden hajoamista sekä kehittämään puhdistusmenetelmiä.

Tässä väitöskirjassa tutkittiin rikkakasvien torjunta-aineiden, atratsiinin ja terbutryynin, hajoamista viidessä boreaaliselta vyöhykkeeltä peräisin olevassa pintamaassa sekä hapen, mikrobien ja maan ominaisuuksien vaikutusta hajoamiseen. Tutkimuksia jatkettiin sedimentti-vesiseoksissa sekä pilot-mittakaavan sedimenttipylväissä. Sedimentit olivat pohjavesialueelta, joka on pilaantunut atratsiinilla, desetyyliatratsiinilla (DEA), simatsiinilla ja 2,6-diklorobenzamidilla (BAM). Tavoitteena oli edistää torjunta-aineiden kemiallista tai mikrobiologista hajoamista fysikaalisella, kemiallisella tai mikrobiologisella käsittelyllä. Fysikaalinen käsittely oli kerran tai kahdesti päivässä toistettu sonikointi, joka kesti 0, 5, 10, 20 tai 30 minuuttia (43 kHz, 320 W). Kemialliset lähestymistavat sisälsivät seuraavien aineiden lisäämisen: pinta-aktiivinen aine metyyli-β-syklodekstriini (0,5 %), nollavalenssisen raudan ja orgaanisen aineksen seos (EHC®) (1 ja 2 %), ja orgaaninen aines eli turve (5 %) tai komposti-turve-hiekka -seos (CPS) (5 ja 15 %). Mikrobiologista näkökulmaa edusti mikrobiologinen hajoaminen sedimentin omien mikrobien toimesta verrattuna steriilikontrolleihin sekä bioaugmentaatio *Pseudomonas* ADP kannalla.

Mikrobit tehostivat atratsiinin ja terbutryynin hajoamista pintamaissa puoliintumisaikojen ollessa 57 - 181 päivää atratsiinilla ja 70 - 291 päivää terbutryynillä. Atratsiini hajosi myös kemiallisesti neljässä maassa, jolloin puoliintumisajat olivat 120 – 183 päivää. Hapella ei ollut yleistä vaikutusta atratsiinin puoliintumisaikoihin, kun taas terbutryyni hajosi vain hapellisissa olosuhteissa. Maassa, jossa hajoaminen oli heikointa, oli vähiten orgaanista ainetta, typpeä, ammoniumia, nitraattia ja nitriittiä. Sonikointi ei laskenut atratsiinipitoisuuksia verrattuna sonikoimattomiin sedimentti-vesiseoksiin ja steriloituihin sedimentti-vesiseoksiin, vaikka mikrobiologisesta hajoamisesta ja sonikoinnin aiheuttamasta kemiallisesta hajoamisesta oli viitteitä. Pinta-aktiivisen aineen lisääminen ei stimuloinut mikrobiologista hajoamista sedimentin omien mikrobien toimesta, ja *Pseudomonas* ADP hajotti atratsiinia myös ilman pinta-aktiivista ainetta. EHC® edisti atratsiinipitoisuuksien laskua kemiallisesti hapellisissa olosuhteissa. EHC® sedimenttipylväissä puhdisti atratsiinilla, DEA:lla ja BAM:lla pilaantunutta pohjavettä verrattuna pylväisiin ilman EHC®:ta, mutta vaikutus kesti vain noin kuukauden. Turve ja CPS tehostivat atratsiinin ja simatsiinin pitoisuuksien laskua kemiallisesti hapesta riippumatta. Turve laski myös heksatsinonin pitoisuuksia. Vain pieniä jäämiä atratsiinia, simatsiinia ja heksazinonia pystyttiin eristämään sedimentistä ja turpeesta, mikä viittaa siihen, että pitoisuuden lasku on johtunut kemiallisesta hajoamisesta ja/tai eristämättömissä olevien sitoutuneiden jäämien muodostumisesta.

Tiivistettynä, turpeen, CPS:n ja *Pseudomonas* ADP:n lisäämiset olivat parhaat lähestymistavat torjunta-ainepitoisuuksien laskemiseksi. Myös EHC® voi olla käytännöllinen pienten torjunta-ainepitoisuuksien (bio)puhdistuksessa, mieluiten hapen läsnä ollessa. On kuitenkin tutkittava lisää turpeen, CPS:n ja EHC®:n käytettävyyttä (bio)puhdistuksessa, jotta vältytään mahdollisilta orgaanisen aineksen lisäämisen haitallisilta vaikutuksilta.

LIST OF ORIGINAL PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. Kerminen, K. & Kontro, M. H. Atrazine and terbutryn dissipation in surface soils from the Boreal Zone in Finland. *Manuscript*.

II. Kerminen, K., Salovaara, V. & Kontro, M. H. 2017. A zero-valent iron and organic matter mixture enhances herbicide and herbicide degradation product removal in subsurface waters. *Journal of Environmental Sciences* 57: 411-417, http://dx.doi.org/10.1016/j.jes.2016.12.013.

III. Kerminen, K. & Kontro, M. H. 2017. Sonication effects on atrazine dissipation in vadose zone sediment slurries. *Environments* 4(1):18, doi:10.3390/environments4010018.

IV. Kerminen, K., Le Moël, R., Harju, V. A., & Kontro, M. H. Enhancing herbicide dissipation in subsurface sediment slurries. *Manuscript*.

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THE AUTHOR'S CONTRIBUTION

I. Corresponding author. KK took samples during the experiment, performed laboratory and data analyses, wrote the first version of the paper, and thereafter co-authored with MHK.

II. Corresponding author. KK and MHK planned the experiment. KK and VS performed the laboratory work, while KK co-authored the manuscript with MHK.

III. Corresponding author. KK and MHK planned the experiment. KK carried out the experiment, performed laboratory and data analyses, and wrote the paper under the supervision of MHK.

IV. Corresponding author. KK and MHK planned the experiments. KK, RLM and VH carried out the experiments. KK did data analyses and wrote the paper together with MHK.

In addition to the results of the original papers, the thesis also includes unpublished additional material analysed by the author.

ABBREVIATIONS

ANOVA	analysis of variance
BAM	2,6-dichlorobenzamide
cfu	colony-forming units
CO_2	carbon dioxide
CPS	compost-peat-sand mixture
EHC®	a commercial product containing zero-valent iron and organic matter
EU	European Union
DEA	desethylatrazine
DEDIA	desethyldesisopropylatrazine
DIA	desisopropylatrazine
dw	dry weight
GC-MS	gas chromatograph-mass spectrometer
HPLC	high performance liquid chromatography
HYA	hydroxyatrazine
K_{oc}	organic carbon partition coefficient
K-W	Kruskal-Wallis test
M-W	Mann-Whitney test
PAH	polyaromatic hydrocarbon
RMA	repeated measures analysis of variance
rpm	revolutions per minute
S.D.	standard deviation
TOC	total organic carbon
Tukey HSD	Tukey's honestly significant difference test
UV/VIS	ultraviolet/visible light

1. INTRODUCTION

1.1 Pesticides and their occurrence in the environment

Pesticides are used to eliminate weeds (herbicides), pest insects (insecticides), fungal diseases (fungicides) and other unwanted biota to protect crops, buildings, humans and domestic animals from harmful effects caused by the pest organisms. The use of pesticides is not limited to agriculture. They are also used in urban areas, e.g. in parks and railroads. As much as 395 944 tonnes of pesticides were sold in the European Union (EU) in 2014 (Eurostat 2016). The widespread use of pesticides has led to their occurrence in the environment. The herbicide atrazine has been found in surface and ground waters, air, rain, soils and subsoils (Lazorko-Connon & Achari 2009, Table 1), often at trace concentrations, but also at higher concentrations as point-source contamination. Even low, ecologically relevant concentrations may affect biota, e.g. if the pesticide such as atrazine acts as an endocrine disruptor (Hayes et al. 2002, Vandenberg et al. 2012). Humans are protected from the harmful effects of drinking-water contamination by legislation. In the EU, the limit value for a single pesticide or degradation product is 0.10 μg l^{-1} and for several 0.50 μg l^{-1} (EU 2006). The limit value was exceeded in 8 % of the ground water areas examined in Finland (Vuorimaa et al. 2007), and in 45 % of groundwater samples in the United Kingdom (Pearson 2006), suggesting that these groundwaters cannot be used without treatment.

1.2 Triazine and benzonitrile herbicides and degradation products

Atrazine [6-chloro-N-ethyl-N´-(1-methylethyl)-1,3,5-triazine-2,4-diamine; Fig. 1] is an herbicide used to control grassy and broadleaved weeds, especially in corn fields, but it has also been used in citrus and nut orchards, coniferous forests, pastures, ornamental ponds, residential and industrial areas (Lazorko-Connon & Achari 2009). Atrazine affects susceptible plants by inhibiting photosynthesis. It is moderately water-soluble, slightly volatile and moderately lipophilic (Lazorko-Connon & Achari 2009, Table 2). Atrazine has several degradation products, e.g. hydroxyatrazine (HYA), desethylatrazine (DEA), desisopropylatrazine (DIA) and desethyldesisopropylatrazine (DEDIA) (Mudhoo & Garg 2011). DEA [2-amino-4-chloro-6-(isopropylamino)-1,3,5-triazine; Fig. 1] has high water solubility and lower affinity for organic carbon than atrazine (Aelion & Mathur 2001, Table 2). The organic carbon-water partition coefficient (K_{oc}) of DEA was 80 ml g^{-1}, while the K_{oc} of atrazine was 140 ml g^{-1} in

Table 1. Atrazine concentrations detected in the environment.

Environment	Concentration	Reference
Agricultural soil	8.3–15.2 μg kg^{-1}	Jablonowski et al. 2010
Soil at dealership sites	0.11–681 mg kg^{-1}	Kruger 1996
Subsoil, groundwater sediment	12.2–14.0 μg kg^{-1}	Mattsson et al. 2015
Streams, rivers, lakes	0.1–47 μg l^{-1}, [a]	Maloschik et al. 2007
Rivers	6.9–224 μg l^{-1}	Lazorko-Connon & Achari 2009
Groundwater	0.01–0.17 μg l^{-1}	Hildebrandt et al. 2007
Groundwater	<0.001–0.34 μg l^{-1}	Vuorimaa et al. 2007
Municipal wells	0.05–2 μg l^{-1}	Kolpin et al. 1998
Groundwater at dealership sites	0.37–4 600 μg l^{-1}	Kruger 1996
Snow, fog, rain	0.03–154 μg l^{-1}	Lazorko-Connon & Achari 2009

[a] may include other pesticides

sediment, which had 1.3 % organic carbon. DEA and atrazine are both easily leachable and are often found in groundwaters (Kolpin et al. 1998, Hildebrandt et al. 2007, Vuorimaa et al. 2007).

Terbutryn [N-(1,1-dimethylethyl)-N'-ethyl-6-(methylthio)-1,3,5-triazine-2,4-diamine; Fig. 1] has been used as pre- and post-emergent herbicide on cereals, sugar cane and maize and in aqueous environments to control the growth of algae and vascular plants, because it inhibits photosynthesis (Tomlin 2000). It is also used in building façade coatings, from which it leaches with rain (Burkhardt et al. 2012). In soil, terbutryn has lower leachability than atrazine, because its K_{oc} is higher (Table 2).

Simazine (6-chloro-N,N´-diethyl-1,3,5-triazine-2,4-diamine; Fig. 1) is a triazine herbicide used to control broad-leaved weeds and annual grasses in citrus, vine, maize, pea, coffee, tea and sugarcane cultivations (Tomlin 2000). It also inhibits electron transport in photosynthesis. Simazine is almost comparable to atrazine in water solubility, volatility, affinity for organic carbon and structure (Table 2, Fig. 1).

Propazine [2-chloro-4,6-bis(isopropylamino)-s-triazine] is another triazine similar to atrazine in structure (Table 2, Fig. 1). It is used to control broadleaved and grass weeds in sorghum, carrot and parsley fields as well as in glasshouse ornamentals (Tomlin 2000).

Hexazinone [3-cyclohexyl-6-(dimethylamino)-1-methyl-1,3,5-triazine-2,4(1H,3H)-dione; Fig. 1] is used to control annual and biennal weeds, e.g. in the cultivation of alfalfa, sugar cane and coniferous species (Tomlin 2000). Hexazinone is very water-soluble and has a tendency to leach (Table 2).

Dichlobenil (2,6-dichlorobenzo-

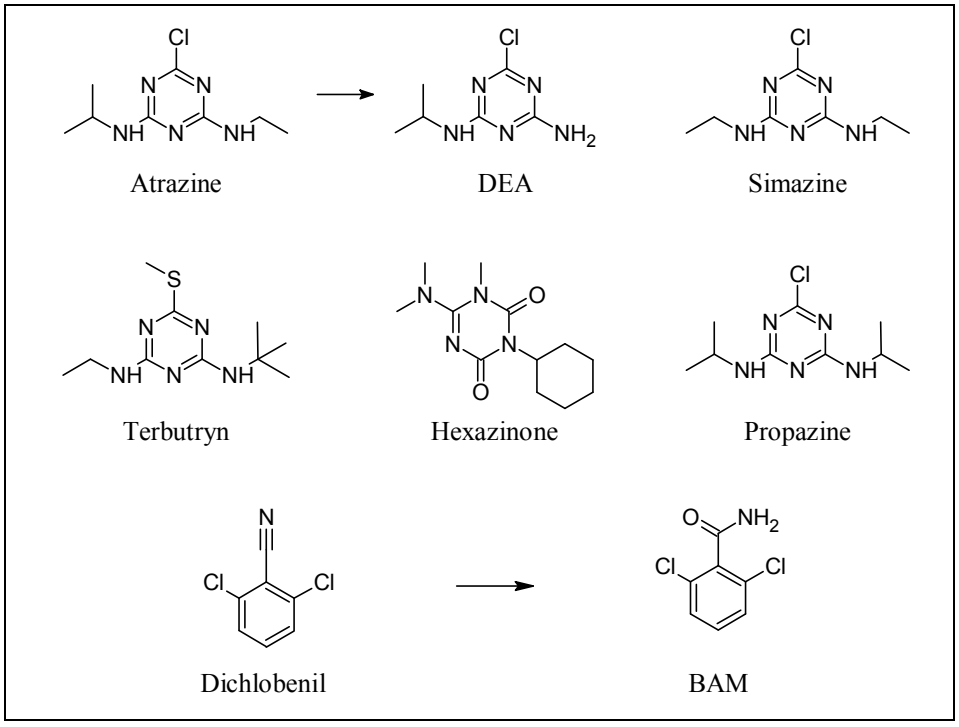

Figure 1. Structural formulas of the pesticides and their degradation products.

Table 2. Pesticide and degradation product properties (Tomlin 2000, unless otherwise mentioned).

Name	Solubility in water (mg l^{-1})	Henry's constant (Pa m^3 mol^{-1})	K_{oc} (ml g^{-1})
Atrazine	33	1.5×10^{-4}	39-173
DEA	3 200[a]		80[a]
Terbutryn	22	1.5×10^{-3}	2000
Simazine	6.2	5.6×10^{-5}	103-277
Hexazinone	33 000		10-54[b]
Propazine	5.0	1.79×10^{-4}	65-268
Dichlobenil	14.6	1.10	500-896[c]
BAM	2 700[d]	1.22×10^{-9}[c]	33-35[c]

[a] Aelion & Mathur 2001
[b] Sarmah et al. 2004, Poulier et al. 2015
[c] Holtze et al. 2008
[d] Geyer et al. 1981

nitrile; Fig. 1) is a benzonitrile herbicide that inhibits cell-wall biosynthesis (Tomlin 2000). It has been used in fruit orchards, forest plantations and public green areas to control annual and perennial weeds. It has also been used to control the growth of aquatic plants. Its degradation product, 2,6-dichlorobenzamide (BAM; Fig. 1), is less volatile and more likely to leach than the parent compound (Table 2).

1.3 Environmental fate of pesticides

Ideally, pesticides would serve their purpose and then disappear from the environment. In practice, pesticides are found in various environmental compartments after use (Table 1). What happens to the pesticide in surface soil affects how likely it leaches to lower soil layers and surface and ground waters. Pesticides undergo sorption-desorption, degradation, transport and sometimes bioaccumulation (Navarro et al. 2007). They can also volatilize or drift as spray or on particles through the air (Dubus et al. 2000). There are many factors that affect the fate of pesticides in the environment. These include weather and climate conditions (Kookana et al. 2010), agricultural practice (Ostrofsky et al. 1997), pesticide properties (Table 2) and soil properties (Lewis et al. 2016). For example, atrazine sorption and degradation were higher in surface soils with higher organic matter content (half-life 37 d) than in lower soil layers (as long as 223 d), where atrazine was highly persistent (Jenks et al. 1998).

Sorption

Organic matter as well as mineral fraction, e.g. clay, can adsorb herbicides (Mudhoo & Garg, 2011). Herbicide properties and soil constituents affect the relative importance of organic and inorganic fractions on sorption. Uncharged organic compounds sorb predominantly to organic carbon, when soil total organic carbon (TOC) content is over 0.1%. Organic matter can be in solid form or dissolved in water. Sorption to soil organic matter can decrease leaching of herbicides. However, complex formation between herbicides and dissolved organic matter or colloids can facilitate transport through soil profile.

Degradation

Mineralization, i.e. complete degradation to carbon dioxide (CO_2), is the only

process that actually removes pesticides from the environment. When the parent compound disappears and is no longer detected, it may still be present as degradation products or bound residues. Under field conditions, it may have also leached down the soil profile. When the word 'dissipation' is used to express decrease in pesticide concentration, it includes the possibility of mineralization, degradation, sorption and/or leaching. Degradation implies, that the parent compound has broken down into smaller units (degradation products / metabolites) and possibly even to CO_2, which is a sign of mineralization.

Degradation of pesticides can involve both abiotic and biotic processes (Fenner et al. 2013). Examples of abiotic degradation include indirect phototransformation and clay-catalysed hydrolysis of atrazine. Organic matter was long considered to catalyse the chemical hydrolysis of atrazine to HYA, i.e. dechlorination, but in 1993 it was shown that microbes can dechlorinate atrazine, and the sterility of sterile controls in previous studies and thus chemical HYA formation in soil was questioned (Mandelbaum et al. 1993). Since then, many atrazine-degrading microbes have been isolated, e.g. *Pseudomonas* Migula sp., *Rhodococcus* Zopf sp., *Ralstonia* Yabuuchi et al. sp., *Rhizobium* Frank sp., *Agrobacterium radiobacter* (Beijerinck & van Delden) Conn and *Ensifer* Casida sp. (Ralebitso et al. 2002, Udiković-Kolić et al. 2012, Ma et al. 2017). *Pseudomonas* sp. strain ADP is probably the best-known atrazine degrader, since its catabolic enzymes and genes (*atzABCDEF*) and degradation pathway have been determined, and it is known to mineralize atrazine (Fig. 2, Singh & Singh 2016). The degradation begins with dehalogenation and is followed by N-dealkylation, deamination and ring cleavage (Ralebitso et al. 2002). Atrazine can also be dechlorinated by a *trzN*-encoded triazine hydrolase enzyme found in *Nocardioides*

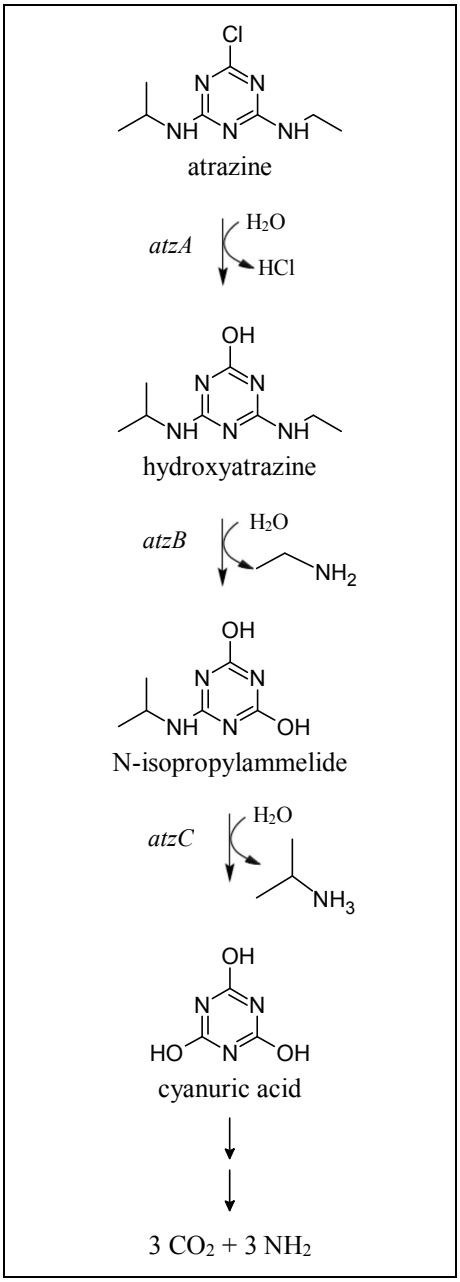

Figure 2. The first three steps of the atrazine catabolic pathway in *Pseudomonas* sp. strain ADP, encoded by the *atzABC* –genes (de Souza et al. 1998, Ralebitso et al. 2002).

Prauser sp. strain C190 (Mulbry et al. 2002). Some strains initiate degradation by N-dealkylation of the isopropyl and ethyl side chains, forming DIA and DEA (Singh & Singh 2016). Dealkylation of atrazine has been associated with the cytochrome P-450 system in *Rhodococcus* sp. strain NI86/21 (Nagy et al. 1995). The atrazine degradation capacity of soil does not always originate from single bacteria, but can also be divided among different members of the microbial community (Singh & Singh 2016). The ability of the microbes and enzymes to degrade different triazines also varies. *Nocardioides* sp. strain C190 degrades triazines with chloro- and methylthio-group (e.g. atrazine, simazine, propazine and terbutryn), while *Pseudomonas* ADP does not degrade methylthio-triazines (Topp et al. 2000).

1.4 Remediation

Contamination of soils and waters has led to the development of remediation techniques. *In situ* techniques are used to clean soils or waters at the contaminated site, while *ex situ* techniques remove the contaminated soil or water and treat it elsewhere (Vidali 2001). Supplying air and nutrients to soil (bioventing) or air to groundwater (biosparging) to stimulate contaminant degradation by indigenous bacteria are examples of *in situ* techniques. Permeable reactive barriers are also built *in situ* in the contaminated groundwater area to clean the water that flows through (Obiri-Nyarko et al. 2014). *Ex situ* techniques involve excavation or pumping of the contaminated substance (Vidali 2001). The treatment can occur in bioreactors or composts or by landfarming.

Remediation can take advantage of abiotic and biotic processes. For example, natural attenuation of contaminants can occur through abiotic processes, such as volatilization and sorption and/or by biodegradation (Mulligan & Yong 2004). Active abiotic remediation can include the use of advanced oxidation processes, such as sonochemical, photochemical and electrochemical oxidation processes, in which hydroxyl radicals are formed (Rodrigo et al. 2014). Another example of abiotic processes is atrazine dechlorination by zero-valent iron (Kim et al. 2008). It is also utilized in a commercial product with organic matter and zero-valent iron (EHC®) that is used to dechlorinate organochlorine pesticides and solvents (Seech et al. 2008).

Bioremediation is remediation carried out by microbes, plants or other living organisms. Degradation by indigenous microbes can be enhanced by biostimulation, i.e. by altering the conditions so that they are favorable for the degraders and degradation. This can include the addition of nutrients, oxygen or other electron acceptors, and varying the moisture, pH and temperature (Vidali 2001). If bioavailability limits biodegradation, the use of surfactants may help (Singh & Cameotra 2014, Mao et al. 2015, Simpanen et al. 2016). When the degradation capacity of the indigenous microbes is limited or even absent, microbes that can degrade the contaminant can be added. This is called bioaugmentation.

2. AIMS OF THE STUDY

The motivation behind this study was to explore ways to clean soil and groundwater contaminated with pesticides. Groundwater is important drinking water source in Finland. Herbicide dissipation was first examined in surface soils from the Boreal region to enlighten factors affecting dissipation in boreal soils. Herbicide dissipation was further studied in subsurface sediment slurries and columns that received sediments from a pesticide-contaminated groundwater area. The purpose was to estimate remediation potential in groundwater zone or in (bio)reactor. The approaches for enhancing herbicide dissipation were physical, chemical and microbiological treatments. The physical treatment was sonication and the chemical treatments included the additions of organic matter [peat and a compost-peat-sand (CPS) mixture], surfactant (methyl-β-cyclodextrin) or a zero-valent iron – organic matter mixture (EHC®). The microbiological aspect included examining degradation by indigenous soil and sediment microbes compared with sterilized controls and bioaugmentation with *Pseudomonas* ADP.

The specific aims were to study:

1. the half-lives of atrazine and terbutryn in five surface soils from the Boreal region, and whether microbes, oxygen and soil properties affect degradation,
2. the usability of EHC® in cleaning groundwater contaminated with atrazine, DEA and BAM and in atrazine dissipation enhancement in vadose zone sediment slurries,
3. the effects of sonication on atrazine dissipation in vadose zone sediment slurries,
4. whether surfactant addition to vadose zone sediment slurries can increase atrazine degradation by indigenous microbes or by *Pseudomonas* ADP,
5. whether organic matter addition enhances the biotic or abiotic dissipation of atrazine, simazine, hexazinone, dichlobenil and BAM in subsurface sediment slurries.

3. MATERIALS AND METHODS

3.1 Surface soils, subsurface sediments and groundwater

The surface soils and subsurface sediments used in the experiments were collected in the city of Lahti (Finland), which is located in the Boreal region (Fig. 3). The sampling sites, apart from flowerbed G, were situated in a groundwater area contaminated with atrazine, DEA, DIA, simazine and BAM (railway C and B in Talja et al. 2008, railway C and garden F in Mattsson et al. 2015). Simazine had been used in flowerbed G and garden F two years before collecting the top 20 cm of the surface soils with a small drill (ø 3 cm). The vadose zone sediments used in the slurries in flasks, were from 11.3 m to 14.6 m deep near the railway station (C in Fig. 3), just above the groundwater table at about 15 m. The combined sediments used in the column experiment were from drillings near the railway station (railway C, 6.1–55.0 m deep, water table at 15 m) and in the city garden (garden F, 18.6–31.0 m deep, water table at 4.6 m). The surface soils and subsurface sediments were stored in plastic bags at +4 °C until use. The groundwater was from Lahti and was contaminated with atrazine (0.08 µg l^{-1}), DEA (0.03 µg l^{-1}) and BAM (0.02 µg l^{-1}).

Figure 3. Sampling sites of railway B and C, garden E and F and flowerbed G soils in Lahti, Finland.

3.2 Design of the experiments

The variables used in the study are summarized in Box 1. Surface soils were used to study factors that may affect herbicide dissipation: oxygen, microbes and soil properties. Dissipation in groundwater deposit slurries was studied in Talja et al. (2008). Subsurface sediment slurries were used to examine the effects of various treatments on herbicide dissipation with the aim to model groundwater environment or slurry (bio)reactor in laboratory-scale. The purpose of the pilot-scale subsurface sediment columns with groundwater flow was to simulate remediation in groundwater zone. Herbicides tend to be more persistent in subsurface soils and groundwater, where there is also often less organic matter. Therefore, the effect of organic matter additions on herbicide dissipation was examined. EHC® was used to examine the combined effects of organic matter and zero-valent iron. Bioavailability may limit herbicide degradation, whereas sonication and surfactants may release herbicides from surfaces and, thus, increase dissipation. The role of microbes in herbicide degradation is important. Therefore, the effects of indigenous sediment microbes and a known atrazine degrader *Pseudomonas* ADP on herbicide dissipation were examined. Both aerobic and anaerobic conditions were studied, because conditions can become anoxic in soils and groundwater.

The treatments in the experiments and the initial concentrations of the compounds studied are presented in Table 3. In the beginning of the experiments, the soils and sediment slurries were spiked with herbicides and degradation products. Atrazine and terbutryn dissipation in five surface soils was examined in 100-ml glass flasks with 15 g dry weight (dw) of moist soil in each flask. The sediment slurries consisted of 15 g dw sediment and 50 ml sterile distilled water in 100-ml glass flasks. The subsurface sediment columns were 2 m high and 5 cm in diameter and contained 1.5 m sediment and 0.5 m groundwater over the sediments. Groundwater flowed through the columns at velocity typical to natural groundwater. The sediments and groundwater were not spiked, but the groundwater was contaminated with atrazine, DEA and BAM. The number of replicates in all experiments was three or four.

1. System
   - 1.1. Surface soils
   - 1.2. Subsurface sediment slurries
   - 1.3. Subsurface sediment columns + groundwater flow
2. Herbicides / degradation products
   - 2.1. Atrazine
   - 2.2. Terbutryn
   - 2.3. DEA
   - 2.4. BAM
   - 2.5. Simazine
   - 2.6. Hexazinone
   - 2.7. Dichlorobenil
3. Treatment
   - 3.1. Physical
     - Sonication
   - 3.2. Chemical
     - Organic matter
     - Surfactant
     - Zero-valent iron + organic matter
   - 3.3. Biological
     - Native microbes
     - *Pseudomonas* ADP

Box 1. The variables used in the study.

The anaerobic conditions were created in the experimental flasks by placing them in incubation jars with Anaerocult A -reagent (Merck KGaA, Darmstadt, Germany), and Anaerotest (Merck) was used to check that the conditions remained anaerobic. Sterilization was carried out by autoclaving for 1 h at 121 °C and 101 kPa on three successive days. The flasks were incubated in the dark, in a shaker and at

Table 3. Experiments, treatments and initial concentrations of the compounds studied. EHC® is a commercial mixture of zero-valent iron (50%) and organic matter (50%) (PeroxyChem,Philadelphia, PA, USA). Compost-peat-sand (CPS) mixture is a commercial product Musta Multa (Biolan, Kauttua, Finland). The percentages are by weight of sediment.

Experiment (Paper)	Material	Treatments/Factors	Compound(s) studied
Herbicide dissipation study (I)	surface soils	aerobic/anaerobic sterilized/non-sterilized soil (B, C, E, F, G)	atrazine (100 µg g^{-1}) terbutryn (67 µg g^{-1})
EHC®, flask experiment (II)	vadose zone sediment slurries	aerobic/anaerobic sterilized/non-sterilized EHC® (0, 1 or 2%)	atrazine (30 mg l^{-1})
EHC®, column experiment with groundwater flow (II)	subsurface sediment + contaminated groundwater	EHC® (0 or 2%)	atrazine (app. 77 ng l^{-1}) DEA (app. 32 ng l^{-1}) BAM (app. 16 ng l^{-1})
Sonication experiment I (III)	vadose zone sediment slurries	sterilized/non-sterilized sonication (0, 5 or 10 min, once & twice a day)	atrazine (100 mg l^{-1})
Sonication experiment II (III)	vadose zone sediment slurries	sterilized/non-sterilized sonication (0, 20 or 30 min, twice a day)	atrazine (100 mg l^{-1})
Various additives (IV)	vadose zone sediment slurries	aerobic/anaerobic sterilized/non-sterilized CPS (0, 5 or 15%) peat (0 or 5%) β-cyclodextrin (0 or 0.5%) *Pseudomonas* ADP (yes/no)	atrazine (30 mg l^{-1})
Herbicide mixture experiment with organic matter additions (IV)	subsurface sediment slurries	sterilized/non-sterilized aerobic/anaerobic peat (0 or 5%) CPS (0 or 15%)	atrazine (15 mg l^{-1}) simazine (15 mg l^{-1}) hexazinone (25 mg l^{-1}) dichlobenil (20 mg l^{-1}) BAM (20 mg l^{-1})

room temperature (22 ± 2 °C). Before sampling, the soils and sediment slurries were mixed carefully and the particles in the slurries were allowed to settle for at least 15 min. Samples from the slurries were taken from aqueous phase. During the column experiment, 1-l effluent water samples were collected, and at the end of the experiment, sediment samples were collected for extraction.

3.3 Chemical, physical and microbiological analyses

The laboratory analyses used here are presented in Table 4. The pesticide analyses are presented in further detail in the following section.

Analyses of pesticides

Simazine as an internal standard (73.8 µg) was added to the 0.1-g surface soil samples, which were extracted three times with 1.5 ml of methanol-water (3:1, vol:vol). The extracts were combined. Extraction included careful mixing, sonication for 15 min and shaking overnight at 200 revolutions per minute (rpm). The extracts and standards (I) were filtered (0.45 µm) and analysed with high performance liquid chromatography (HPLC), which comprised a Waters sample processor (712 WISP; Waters Copr., Milford, MA, USA), two pumps (model 6000A) and Symmetry C18 column (3.9 x 150 mm), and Hewlett-Packard ultraviolet (UV) detector (HP 1050, 225 nm; Hewlett-Packard, Palo Alto, CA, USA). The mobile phase flowed at 1 ml min^{-1} and consisted of 10 mM phosphate buffer (pH 7.0) and acetonitrile. Acetonitrile concentration was increased from 30 % to 70 % in 12 min, and after 1 min the concentration was decreased to 30 % in 5 min and held there for 5 min.

The 100–500-µl water samples from the slurries were amended with 100 µl of simazine as an internal standard and methanol-water (3:1, vol:vol) to obtain a final volume of 600 µl and internal standard concentration of 5.06–15.3 mg l^{-1}. The standards (II–IV) contained the same concentration of internal standard as the samples. The pesticides were analysed with a Shimadzu Prominence HPLC-UV/VIS (ultraviolet/visible light) apparatus (Shimadzu Corp., Kyoto, Japan) and SunFire C18 column (Waters) (II–IV). The flow rate was 0.4 ml min^{-1} and the mobile phase consisted of acetonitrile and filtered distilled water. For the first 3.5 min acetonitrile concentration was held at 30 %, followed by increase to 65 %, which was held until 8.5 min, and decreased to 30 % until 14 min.

Propazine as an internal standard (Fig. 1, Table 2) was added to the 500 ml water samples from the column experiment, and the samples were filtered (II). The samples were concentrated with solid-phase extraction, evaporated and the precipitates extracted with acetone. The filtered samples were analysed with a gas chromatograph-mass spectrometer (Shimadzu GCMS-QP-2010Ultra) and ZB-5MS capillary column (II).

The pesticides from the subsurface sediments were extracted by adding methanol-water (3:1, vol:vol) and 20 µg (II and III) or 9.96 µg (IV) of propazine as an internal standard to the sediment. The extractions were sonicated for 20 min, shaken overnight at 200 rpm and centrifuged. The supernatants from three extractions were combined and evaporated, and the precipitates were solubilized in methanol-water (3:1) or acetone. The pesticides remaining in the sediments after the experiments were analysed with GC-MS as in II–IV. The pesticides initially occurring in the sediments were analysed using Shimadzu Prominence HPLC-UV/VIS equipment, as presented in Mattsson et al. (2015).

Table 4. Methods used in the thesis.

Analyses	Paper	Reference / method
Chemical and physical analyses		
pH	I, II, IV	I: soil : distilled water -ratio 2 : 5 (vol : vol), pH-meter II: pH from slurry (pH-meter) and effluent (pH paper) IV: slurry pH with pH-meter or pH-paper
dry weight	I, II, III, IV	SFS-EN 13040 (2000) / 105 °C for 16 h
water content of saturated soil	I	soil wetted with distilled water and allowed to stand in funnel for 24 hours
organic matter content	I, II, III, IV	SFS-EN 13039 (2000) / 550 °C for 4 h
grain size distribution	III	III
Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn	I, III	Talja et al. (2008); ISO 11885 (1996), ISO/CD 22036 (2005); Ramboll Analytics
total carbon	I, III, IV	Talja et al. (2008)
total organic carbon (TOC)	II	SFS-EN 1484 (2012)
total nitrogen	I, III, IV	Talja et al. (2008)
ammonium	I, III	Talja et al. (2008): SFS 5505 (1988), Ramboll Analytics
nitrate	I, III	Talja et al. (2008): SFS-EN ISO 10304-1 (1995), SFS-EN ISO 10304-2 (1997); Ramboll Analytics
nitrite	I, III	Talja et al. (2008): SFS-EN ISO 10304-1 (1995), SFS-EN ISO 10304-2 (1997); Ramboll Analytics
pesticide extraction	I, II, III, IV	II; Mattsson et al. (2015)
pesticide analysis by HPLC-UV/VIS	I, II, III, IV	I: Talja et al. (2008); II: Pukkila & Kontro (2014) III: III, Mattsson et al. (2015)
pesticide analysis by GC-MS	II, III, IV	II, III, IV
Microbial analyses		
checking sterility	I, IV	I: plating on Luria-Bertani (L-B) plates with inoculation loop IV: plating on L-B plates
microbial counts	III, IV	III: plating from dilution series (Liu et al. 2015, Pukkila et al. 2009) IV: plating from dilution series on L-B plates

3.4 Calculations and statistical analyses

The results are mostly presented as mean ± standard deviation (S.D.). The herbicide half-lives were calculated, using the equation

$$(1) \qquad \ln C_{(t)} = -kt + \ln(C_0),$$

where the concentration at time t (days) is $C_{(t)}$, the initial concentration is C_0 and k is the rate constant. In the herbicide mixture experiment, the final slow dissipation phase was omitted from the half-life calculations.

The effects of the various treatments and factors on the pesticide half-lives and concentrations were determined with the SPSS Statistical package for Windows (SPSS Inc., Chigago, IL, USA) or IBM SPSS Statistics (IBM Corporation, NY, USA). When the data were normally distributed (Kolmogorov-Smirnov's test $p > 0.05$) and the variances were homogenous (Levene's test $p > 0.05$), parametric analysis of variance (ANOVA) and Tukey's honestly significant difference (Tukey HSD) test were used (II, III). Otherwise, the nonparametric Kruskal-Wallis (K-W) test, and Mann-Whitney (M-W) test for pairwise comparisons were used (I–IV). Repeated measures analysis of variance (RMA) was used to determine the sonication effects, even though at few time points, p was < 0.05 in Levene's test and Kolmogorov-Smirnov's test (III). Each time point was usually analysed separately. Correlations were examined with Pearson's correlation analysis.

4. RESULTS AND DISCUSSION

4.1 Atrazine and terbutryn dissipation in boreal surface soils

The importance of oxygen, microbes and soil properties in atrazine dissipation was examined in five surface soils from the Boreal region. Oxygen did not have an overall impact on atrazine half-lives (K-W, $p > 0.05$), while microbes and soil sites did (K-W, $p \leq 0.001$) (I). Microbes enhanced atrazine degradation in all soils, although not under all conditions. Microbes did not degrade atrazine in the anaerobic railway B soil and aerobic railway C soil, based on the comparison of atrazine dissipation in soils and sterilized soils (Fig. 4). The indication that atrazine degradation can in some soils be limited by the presence or absence of oxygen may be environmentally relevant. For example, heavy rains and flooding could create anaerobic conditions, and hinder atrazine degradation in the railway B soil (I). Since atrazine is commonly found in surface and ground waters (Lazorko-Connon & Achari 2009), clearly not all of atrazine has been degraded in the surface soils, for one reason or another.

The atrazine half-lives in the railway B soil ranged between 181 d and no dissipation, while in the four other soils the half-lives were shorter: 57–183 days (M-W, $p < 0.001$, Table 2 in I). Atrazine was degraded in the railway B soil only in the presence of microbes and oxygen. In the four other soils, the atrazine dissipated chemically below the detection limit in 400 d and more rapidly in the presence of microbes (Fig. 4). The poor microbial and chemical dissipation in the railway B soil

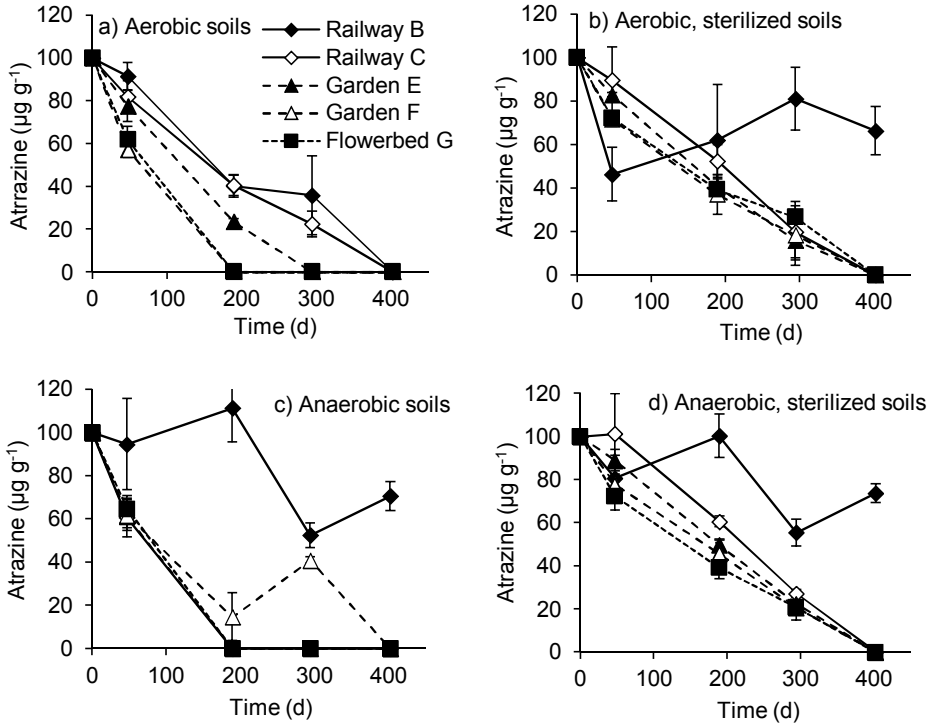

Figure 4. Atrazine concentrations (mean ± S.D., n = 3) in five surface soils under aerobic (a, b) and anaerobic (c, d) conditions, and in sterilized soils (b, d).

could have been associated with the soil composition and nutrient levels. The railway B soil had only 1.0% organic matter, compared with 8.2–16.8% in the other soils. The concentrations of total-carbon, total-nitrogen, ammonium, nitrate, nitrite, and the elements Co, Cr, Mn and Zn were also lowest in the railway B soil (Table 1 in I). However, the inorganic element concentrations were not associated with atrazine half-lives, while total-C ($p = 0.088$), total-N ($p = 0.044$) and NO_3 ($p = 0.041$) correlated with the half-lives in the presence of oxygen.

The atrazine half-lives in the four more organic boreal soils (57–139 d) were similar to the half-lives in temperate soils that had not been adapted to atrazine, i.e. between 28 and 178 d (Accinelli et al. 2001, Krutz et al. 2010). Shorter half-lives have been found in adapted soils in temperate and subtropical regions (Kookana et al. 2010, Krutz et al. 2010). Atrazine dissipation was slower and even non-existent in the railway B soil, which implies that atrazine may persist in some boreal soils. Persistence may be increased by boreal weather conditions. The annual average temperature at the soil collection sites was 4–5 °C in 1981–2010, and the average summer temperature was 15–16 °C, with maximum mean temperatures of 20–22 °C (Finnish Meteorological Institute 2016). However, the experiment was conducted in the laboratory at 22 ± 2 °C. Previously, increase in temperature accelerated atrazine and terbutryn dissipation (Lechón et al. 1997, Kookana et al. 2010, Lewis et al. 2016). Aging of the atrazine residues may also decrease biodegradation (Barriuso et al. 2004). The soils were spiked in the beginning of this experiment, which represents situation right after application. However, the experiment was long.

Terbutryn dissipated below the detection limit in 189–402 d in the aerobic railway C, garden E and F and flowerbed G soils with the aid of microbes (half-lives 70–218 d). Even though the half-life calculations showed no clear chemical dissipation, the terbutryn concentrations in the four aerobic sterilized soils decreased to 46 ± 5 μg g^{-1} from the initial 67 μg g^{-1} (Fig. 5). In the aerobic railway B soil and sterilized B soil, the terbutryn concentration decreased to 35 ± 8 μg g^{-1}, chemically. Terbutryn did not dissipate under anaerobic conditions, apart from a slight decrease to 52 ± 6 μg g^{-1} in the railway B soil and sterilized B soil. In general, terbutryn dissipation seemed to require oxygen and was enhanced by microbes in the four soils with higher organic matter and nutrient content. Muir and Yarechewski (1982) observed also that biological activity and oxygen were important for terbutryn degradation. Terbutryn half-lives in aerobic sediment-water systems were 180–380 d, while under nitrogen-aerated conditions the half-lives were over 650 d (Muir & Yarechewski 1982). In the aqueous phase of boreal groundwater deposit microcosms, microbial terbutryn degradation was only detected in the presence of oxygen with half-lives of 177–349 d (Talja et al. 2008). In a study in Spain by Lechón et al. (1997), terbutryn half-lives in surface soils were mostly as short as 7–58 d and as long as 227 d, and the half-lives were shorter in flask incubations than in packed or intact soil cores. This implies that the half-lives obtained in this study in flasks may have resulted in underestimation of the persistence of terbutryn in intact soil in the environment. Together with cold climate, the possible lack of degraders and occasional anaerobic conditions, terbutryn has the potential to persist in boreal environments. Even in the soils that showed degradation capacity, terbutryn dissipation below the detection limit required at least half a year at room temperature.

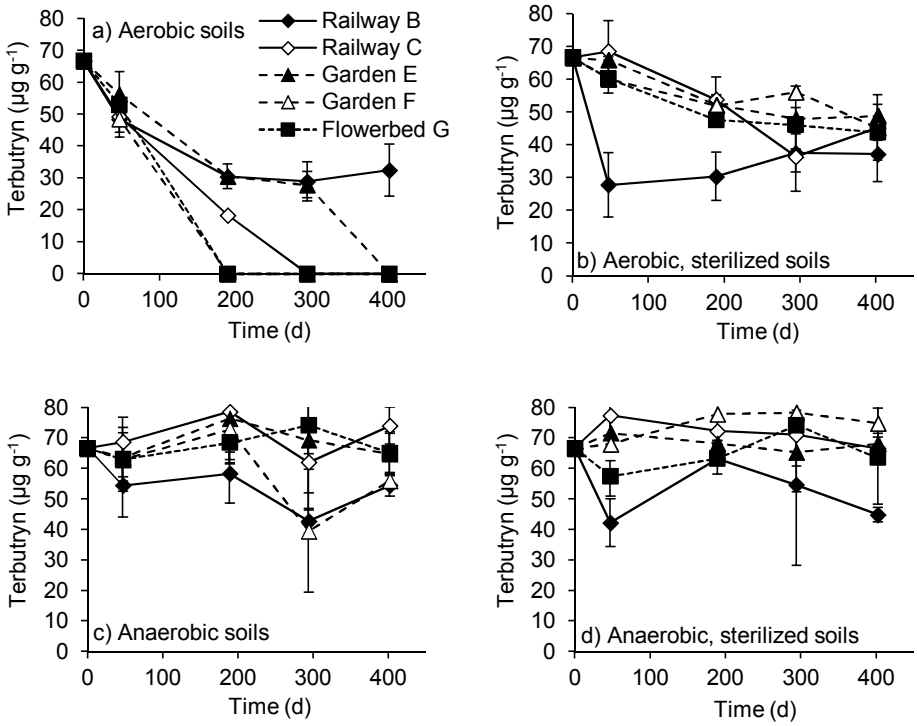

Figure 5. Terbutryn concentrations (mean ± S.D., n = 3) in five surface soils under aerobic (a, b) and anaerobic (c, d) conditions, and in sterilized soils (b, d).

4.2 Effects of a zero-valent iron and organic matter mixture on atrazine, DEA and BAM in subsurface sediment – water systems

The effects of a commercial zero-valent iron and organic matter mixture called EHC® on herbicide dissipation was studied in flasks and in pilot-scale columns. The application of 2.0 % EHC® by mass of sediment into slurries and sterilized slurries enhanced atrazine dissipation, compared with unamended controls under aerobic conditions (Tukey HSD, $p < 0.05$) but not under anaerobic conditions (Fig. 1 in II). At the end of the experiment (day182), 75.0 ± 4.3 % of the atrazine remained in the aerobic slurries with 2.0 % EHC®, and a similar amount (77.8 ± 2.1 %, 23.3 mg l^{-1}) in the corresponding sterilized slurries, which indicates chemical dissipation. The atrazine dissipation efficiency was 0.88 ± 0.14 mg g^{-1} of EHC®.

In the sediment column experiment, groundwater flowing through the columns contained 0.077 ± 0.021 µg l^{-1} atrazine, 0.032 ± 0.0075 µg l^{-1} DEA and 0.016 ± 0.0036 µg l^{-1} BAM. At first, the 2.0 % EHC® decreased the atrazine, DEA and BAM concentrations in the effluent water, compared with the controls without EHC® (M-W, $p < 0.05$, Fig. 2 in II). The effect lasted 33–64 days. The EHC® in the water-saturated sediment columns dissipated about 7.6 µg of the pesticides and degradation products per gram of EHC® (II).

The chemical dissipation induced by EHC® could have resulted from chemical degradation or sorption. Half of the EHC® is zero-valent iron, which has been used to reductively degrade a broad range of organic compounds, e.g.

chlorinated solvents and atrazine (Kim et al. 2008, Tosco et al. 2014). EHC® also contains app. 50 % organic matter, which is known to bind atrazine (Mudhoo & Garg 2011). EHC®-amended sediments from the column experiment were extracted at the end of the experiment, and the atrazine, simazine, DEA, DIA, desethyldeisopropylatrazine (DEDIA) and BAM were not detected. This implies that the compounds studied had transformed into other molecules or had become unextractable.

The dissipation capacity of EHC® seemed rather low and limited, although statistically significant (Fig. 1 and 2 in II). The atrazine dissipation efficiency of 0.88 ± 0.14 mg g^{-1} of EHC®, however, indicates that 1 g of EHC® could decontaminate 8800 l of water containing 0.10 µg l^{-1} atrazine, which is the EU drinking water limit value for a single pesticide (European Union 1998). This estimate was based on results under aerobic conditions. The dissipation capacity was weaker under anaerobic and water-saturated conditions, which implies that aeration may be needed if anaerobic ground waters are remediated with EHC®. The possible adverse effects of EHC® should be considered before applying it in practice. For example, the organic matter source of the commercial product is not known, and TOC was released from the EHC® columns into flowing groundwater, which may have also weakened the dissipation by EHC® (Fig. 2 in II).

4.3 Effects of sonication on atrazine dissipation

The feasibility of enhancing microbiological or chemical atrazine dissipation by sonication was studied in vadose zone sediment slurries and sterilized slurries. The sonications were carried out once and twice a day for 0, 5 and 10 min and twice a day for 0, 20 and 30 min. The atrazine concentrations were significantly lower in slurries than in sterilized slurries in both sonication experiments (RMA, $p < 0.001$; Table 1 in III, Figs. 1 and 2 in III). Anilines and 2,4,6-T (trichlorophenoxyacetic acid) were also less bound in autoclaved than in nonautoclaved soil (Bollag et al. 1978, Koskinen & Cheng 1982). Autoclaving was assumed to alter the soil organic matter and to affect its adsorption sites (Koskinen & Cheng 1982) or to eliminate its biological activity which would transform anilines or their adsorption onto soil (Bollag et al. 1978). Atrazine adsorption was decreased by the presence of dissolved organic carbon and organic matter in solution (Baskaran et al. 1996, Ben-Hur et al. 2003). In addition, chlordane solubility was increased by the presence of humic substances in water (Johnson-Logan et al. 1992). Autoclaving may have released organic matter into the water and increased the solubility and decreased the adsorption of atrazine.

The atrazine concentrations were not affected by once a day sonication for 5 and 10 min (Fig. 1 and Table 1 in III). Twice a day sonication for 10 min increased the atrazine concentrations in sterilized slurries compared with sterilized slurries sonicated for 0 and 5 min (RMA, $p \leq 0.002$). The concentrations did not increase significantly in slurries sonicated for 10 min twice a day, which implies that indigenous sediment microbes had degraded atrazine in the slurries.

Contrasting results were obtained in the second sonication experiment. The atrazine concentrations did not increase in sterilized slurries sonicated twice a day for 20 and 30 min (Fig. 2 and Table 1 in III). In the first experiment, twice a day sonications were initiated on day 25, by which time the atrazine concentrations in the sterilized and sonicated slurries had decreased to 59.3 ± 5.4 mg l^{-1} ($n = 6$), and the twice a day sonications increased the concentrations to 79.6 ± 8.8 mg l^{-1} by day 36. When the sonications were initiated in experiment 2 on day 6, the atrazine

concentrations were still 76.8 ± 7.2 mg l^{-1} (n = 6) in the autoclaved slurries to be sonicated.

While the twice a day sonications for 5 and 10 min did not affect the atrazine concentrations in the slurries, the twice a day sonications for 20 and 30 min increased the concentrations compared with the non-sonicated slurries (RMA, $p \leq 0.002$). The microbes may not have had the time to adapt to degrading atrazine during the 6 d before 20- and 30-min sonications compared with the 25 d before the twice a day sonication for 5 and 10 min. The longer sonication times may also have been harmful for biodegradation. Wood et al. (1997) discovered that continuous sonication for 12 h (36 kHz, 150 W) did not kill *Klebsiella oxytoca* (Flügge) Lautrop strain P2, but prevented growth, cell division and sugar metabolism, while 15-min exposures to sonication at intervals of 240 min increased ethanol production from cellulose. Microbial phenantrene degradation was enhanced by sonication (25-min exposures at intervals of 180 min, 42 kHz, 70 W) only in solid-liquid, not in liquid-liquid systems (Isaza & Daugulis 2009). Slurry particles may have protected the microbes from the harmful effects of sonication to some extent.

The increase in atrazine concentrations by sonication (Fig. 6) could be explained by saturation of the slurries with atrazine. Not all of the atrazine was soluble. Part of it was solid, and some was attached to particles. Normally, the samples were prepared so that the internal standard and then the methanol-water (3:1, vol:vol) were added to obtain a volume of 600 µl. When the samples were filtered through 0.45-µm filters before dilution in methanol-water, the atrazine concentrations were close to the atrazine water solubility of 33 mg l^{-1}, i.e. lower than in the samples first diluted in methanol-water (ANOVA, $p < 0.001$, Fig. 7). It seems, that the samples normally included atrazine that was bound to the

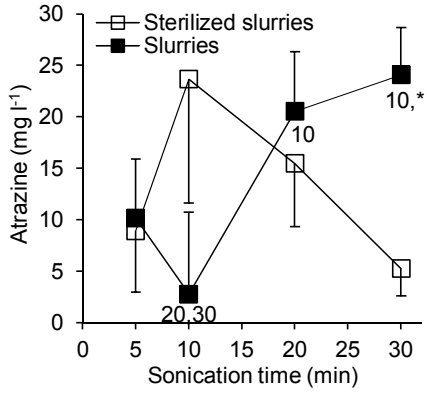

Figure 6. Atrazine increase by sonication, compared with corresponding sediment slurries and sterilized sediment slurries without sonication. The concentrations are average from days 34–36 for the 5- and 10-min sonications, and from day 13 for 20- and 30-min sonications. The asterisk indicates statistically significant differences (Student's t-test, $p < 0.050$) between the slurries and sterilized slurries. The numbers indicate statistically significant differences (ANOVA, Tukey HSD, $p < 0.050$) between the sonication times.

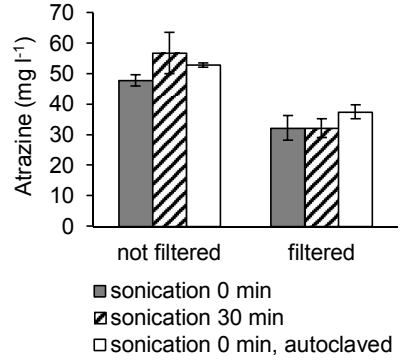

Figure 7. Release of atrazine from particles and aggregates. The 'not filtered' samples were diluted in methanol-water (3:1, vol:vol) before filtering for HPLC. The 'filtered' samples were filtered prior to dilution in methanol-water through 0.45-µm filters. The samples were from sonication experiment II, day 111.

particles or aggregates larger than 0.45 µm, released into the methanol. Sonication may have distributed these more evenly in water and released atrazine from sediments.

Sonication for 5, 10, 20 or 30 min once or twice a day at 43 kHz and 320 W in a sonication bath did not decrease the atrazine concentrations below that of the non-sonicated controls, i.e. sonication enhanced neither abiotic nor biotic atrazine dissipation. The sonication horn seemed more efficient at atrazine degradation than the sonication bath, since as much as 75% of the atrazine in the slurry was degraded by a 10-min sonication at 20 kHz and 150 W with the sonication horn (Collings & Gwan 2010, Table 2 in III). Chemical atrazine degradation was enhanced when sonication was combined with other advanced oxidation processes, such as photolysis, photocatalysis and ozonation (Bianchi et al. 2006, Bahena et al. 2008, Xu et al. 2014). Using the sonication horn instead of the bath and finding the right sonication parameters could make sonication a useful method for cleaning atrazine contamination, especially combined with other oxidation methods. Slurry reactors could be a useful application of the sole use of sonication, since as much as 2.5 tonnes of contaminated soil slurry could be treated in a pilot plant in a day (Collings et al. 2010). *In situ* application of sonication could be impractical and also risky, since sonication may result in desorption and therefore increase the mobility of the pesticides.

4.4 Use of surfactants in atrazine remediation

Surfactants have been used to desorb organic contaminants from soil, which has enhanced their biodegradation (Mao et al. 2015, Simpanen et al. 2016). Here, indigenous sediment microbes appeared to degrade atrazine only, when a high concentration of 100 mg l^{-1} was used, compared with the 20- and 30-mg l^{-1} concentrations. Microbial degradation apparently ceased at 63.2 mg l^{-1} in the sonicated slurries, which is a rather high concentration (III). We assumed that atrazine bioavailability may be limited by its interactions with slurry particles, because atrazine and inorganic/organic colloids form complexes (Meng & Carper 2000, de Jonge et al. 2004). However, adding 0.5% levels of the surfactant methyl-β-cyclodextrin to slurries with 30 mg l^{-1} atrazine did not stimulate degradation by indigenous microbes (Fig. 1 in IV), although atrazine solubility was increased 1.5-fold by β-cyclodextrin (de Carvalho & de Matos Alves Pinto 2012). In addition, *Pseudomonas* ADP decreased atrazine concentrations below 0.5 mg l^{-1} equally in the slurries with and without cyclodextrin (Fig. 1 and Table 2 in IV), which implies that bioavailability did not limit degradation by this strain at the concentrations used. Atrazine bioavailability and different surfactant concentrations should be examined and possibly lower atrazine concentrations used to assess whether methyl-β-cyclodextrin could improve the bioavailability and biodegradation of atrazine. However, the surfactant may be more useful in bioremediation of more hydrophobic compounds. Simpanen et al. (2016) found that methyl-β-cyclodextrin was more beneficial in desorption and removal of larger polyaromatic hydrocarbon (PAH) compounds with four or five aromatic rings than PAHs with two or three rings. The surfactants triton X-100 and rhamnolipids have, however, increased atrazine degradation in soil by *Acinetobacter* Brisou & Prévot sp. strain A6 (Singh & Cameotra 2014). The positive effect was observed only, when surfactant concentration was over critical micelle concentration, i.e. micelles were formed.

4.5 Organic matter additions to sediment slurries

Addition of peat and a compost-peat-sand (CPS) mixture to vadose zone sediment slurries decreased the atrazine concentrations significantly (K-W, $p < 0.001$, Fig. 1 in IV, Table 2). The atrazine concentrations decreased to 3.14 ± 1.89; 0.69 ± 0.47 and 0.06 ± 0.02 mg l^{-1} (mean ± S.D.) in all the slurries and sterilized slurries amended with 5% and 15% CPS and 5% peat, respectively. Peat decreased the concentrations the most, while oxygen did not affect the concentrations. Microbial degradation was not detected, since the sterilized slurries did not differ from the slurries with indigenous microbes. In the end of experiment, some sterilized slurries were contaminated, but their atrazine concentrations did not differ from those in the non-contaminated sterilized slurries. Therefore, the dissipation was assumed to be chemical, i.e. sorption and/or chemical degradation. Organic matter is known to bind atrazine (Dunigan & McIntosh 1971), and adsorption would remove atrazine from the aqueous phase of the slurries. Chemical atrazine degradation in soils has been reported before, even though the dissipation was mostly caused by binding to the soil, and atrazine was not mineralized in the sterilized soil (Kruger et al. 1997, Miller et al. 1997).

Another experiment with atrazine, simazine, hexazinone, dichlobenil and BAM revealed that peat decreased the atrazine, simazine and hexazinone concentrations and half-lives significantly in slurries and sterilized slurries, regardless of the presence or absence of oxygen (K-W, M-W, $p < 0.001$, Fig. 2 in IV, Table 3). CPS decreased the atrazine and simazine concentrations and half-lives (M-W, $p < 0.001$, Fig. 2 in IV, Table 3). The dichlobenil concentrations decreased even without organic matter additions. BAM showed only slight or no dissipation. After the experiment, the liquid phases of the slurries were removed and the pesticides extracted from the sediment and peat. The extracted amounts corresponded to 0.02 ± 0.01 µg l^{-1} of atrazine, 0.04 ± 0.02 µg l^{-1} of simazine and 156.1 ± 72.7 µg l^{-1} of hexazinone. This suggests that the dissipation was caused by chemical degradation. The formation of unextractable bound residues can also decrease pesticide concentrations (Gevao et al. 2000), and it has been an important abiotic dissipation route for atrazine (Kruger et al. 1997, Miller et al. 1997).

The significant decrease in atrazine and simazine concentrations implies that peat and CPS could have potential for use in remediation. The application should be carefully planned, however, because the release of organic matter from peat and CPS could pose a risk to groundwater quality. CPS also contains chicken manure, which cannot be applied to drinking-water resources. Pure peat would be better than CPS in remediation. Peat is already used as a biobed component on farms, because it provides sorption capacity, abiotic pesticide degradation and moisture control (Del Pilar Castillo & Torstensson 2007). Biobeds are layers of biomixture, e.g. straw, peat and soil, on top of a clay layer, and their purpose is to hold and degrade pesticides that have spilled during spraying equipment cleaning and filling. Other potential uses of peat, e.g. in permeable reactive barriers, and their risks should be examined, as well as its role in sorption and degradation processes.

4.6 Microbial degradation

Microbial atrazine degradation was detected in all surface soils, and terbutryn degradation in four soils out of five. There were also indications of microbial degradation in the subsurface sediment slurries sonicated for 10 min twice a day, and the microbial numbers were higher in slurries with 100 mg l^{-1} atrazine [$>10^8$

colony-forming units (cfu) ml^{-1}] than in slurries without atrazine (7×10^4 cfu ml^{-1}). However, atrazine degradation could not be stimulated by adding surfactant, peat or CPS, and microbial degradation was similarly not detected in the non-amended controls. The right conditions for stimulating degradation may simply not have been found. Previously, Nousiainen et al. (2014) found low copy numbers of atrazine degradation genes in boreal subsoil, even though atrazine mineralization was lacking. Biodegradation was stimulated by adding a carbon source (molasses/ citrate) at 10 °C but not at 30 °C (Nousiainen et al. 2015), i.e. in addition to the nutrients chosen, temperature could also have affected biostimulation success. The presence and activity of degraders is necessary for biodegradation.

Pseudomonas ADP significantly degraded atrazine in slurries (M-W, $p < 0.001$, Fig. 1 in IV). It has been suggested that this strain could be useful in remediation of atrazine-contaminated aquifers that lack intrinsic degradation capacity (Shapir et al. 1998). The addition of sodium citrate may be needed or beneficial in supporting survival of the strain among the indigenous microbial community (Mandelbaum et al. 1995, Silva et al. 2004, Nousiainen et al. 2015), even though in our study citrate was not needed for degradation by *Pseudomonas* ADP. Repeated inoculations also enhanced survival and activity of the inoculated strain (Newcombe & Crowley 1999).

5. CONCLUSIONS

Microbes were important for atrazine degradation in surface soils from the Boreal region, although atrazine also dissipated chemically below the detection limit in four soils out of five. Soil with the lowest amounts of organic matter (1.0 %), carbon, nitrogen, nitrate and nitrite showed the poorest microbial and chemical degradation. Oxygen did not have an overall effect on atrazine half-lives, although microbial atrazine degradation in one soil was dependent on oxygen. Terbutryn degradation was best in the presence of microbes and oxygen.

Application of 2.0 % EHC® reduced atrazine concentrations chemically 22% to 23.3 mg l^{-1} in the aerobic, but not in the anaerobic slurries. In water-saturated sediment columns, EHC® decreased the atrazine, DEA and BAM concentrations in groundwater (0.016–0.077 µg l^{-1}) below detection for about 1 month. EHC® can probably be used to clean water contaminated with small quantities of the compounds studied, preferably in the presence of oxygen.

Sonication either increased the atrazine concentrations or did not affect them. Sonication did not decrease the concentrations compared with the non-sonicated treatments, which implies that the sonication conditions used did not enhance the microbiological or chemical dissipation of atrazine. However, there was some microbial degradation in slurries sonicated for 10 min twice a day. Enhancing microbial atrazine degradation by sonication was difficult, however. There was also an indication of chemical dissipation in sterilized slurries sonicated for 30 min twice a day. Together with published evidence, it can be concluded that sonication can induce and enhance chemical atrazine degradation. Combining sonication with some other treatment methods could be beneficial. For example, it would be interesting to determine whether the usability of EHC® could be prolonged by sonication.

Atrazine biodegradation by indigenous sediment microbes could not be stimulated with surfactant methyl-β-cyclodextrin, with the aim of increasing atrazine bioavailability. The right surfactant concentration may not have been used, and the surfactant may be more useful in bioremediation of more

hydrophobic contaminants. *Pseudomonas* ADP degraded atrazine even without cyclodextrin. Other surfactants have also increased atrazine biodegradation.

Peat and CPS decreased the concentrations of atrazine and simazine significantly to 0.03–4.4 mg l^{-1}, while peat also decreased the hexazinone concentrations to 10.5–13.8 mg l^{-1}. The dissipation was chemical rather than microbial and was caused by chemical degradation and/or unextractable bound residue formation.

Attempts to stimulate degradation by vadose zone sediment microbes mostly failed, even though there were indications of microbial degradation in the sonication experiment. The right conditions for activating degradation may not have been found or the sediments lacked degradation capacity. *Pseudomonas* ADP decreased the atrazine concentrations significantly to 0.04–0.5 mg l^{-1}, which implies that this strain could be useful in bioremediation, at least at high atrazine concentrations.

Of the treatment methods examined, the addition of peat, CPS and *Pseudomonas* ADP seemed to be the most promising. Peat and CPS should be further studied to gain a better understanding of sorption and degradation processes. Their applicability in remediation of pesticide-contaminated soils, subsoils and groundwater should also be determined. Possible adverse effects to groundwater quality should be examined as well as dissipation/degradation at low, environmentally relevant concentrations.

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