Potential of the slow pyrolysis products birch tar oil, wood vinegar and biochar in sustainable plant protection
- pesticidal effects, soil improvement and environmental risks

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THE AUTHOR’S CONTRIBUTION

I. MH, TP and HS planned and established the “garden experiments” with *A. arbustorum*. MH planned and performed the microcosm experiment with snails. MH and IL wrote the manuscript under the supervision of HS, OPP and KT.

II. Corresponding author. MH planned and established the “garden experiment”, performed the laboratory and data analyses. MH conducted the laboratory analyses of the “field experiment” with TP. MH carried out the toxicity tests with *Aporrectodea caliginosa* and *Folsomia candida*. MH wrote the manuscript under supervision of HS and OPP.

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IV. Corresponding author. The study was planned by KT, HS and MH. MH set up, maintained and was responsible for the sampling and analysis of the experiment. MH performed data analysis and wrote the manuscript under supervision of HS and OPP.

In addition to the results of the original papers, the thesis also includes unpublished additional material analysed by the author.

ABSTRACT

The increased use of pesticides and their impacts on terrestrial and aquatic environments have become a matter of considerable concern in recent decades. The use of pesticides, especially synthetic ones, is suggested to be replaced by compensatory substances that exert a lower risk to the environment. The risks caused by residues and degradation products of pesticides in soils and aquatic systems should also be reduced. At the same time, global climate change will lead to an increase in temperature and rainfall in some areas, which could enhance the growth of several pest and weed populations. For example, populations of the land snail (*Arianta arbustorum*) and the Iberian slug (*Arion lusitanicus*) have substantially increased in many parts of northern Fennoscandia in recent years. Consequently, these molluscs have rapidly become an increasing problem with severe impacts, particularly in private gardens.

Plant-derived products may have a significant role in sustainable plant protection when functioning as compensatory substances for synthetic pesticides, or alternatively by affecting the behaviour of synthetic pesticides in the soil. This thesis research investigated the suitability of birch (*Betula sp.*) -derived slow pyrolysis products, birch tar oil, wood vinegar and biochar, in sustainable plant protection. The aims of the study were to (i) explore the efficiency of birch derived pyrolysis liquids in mollusc control and (ii) investigate the environmental risks related to their use. In addition, (iii) the effects of biochar and wood vinegar on the environmental fate of glyphosate, the most common herbicide used against a wide range of weeds in Finland, was examined.

Birch tar oil and wood vinegar proved to be ineffective in eliminating snails. Instead, birch tar oil and the mixture of birch tar oil and wood vinegar exhibited a clear repellent effect against snails and slugs. The effect of wood vinegar on non-target organisms was assessed in several toxicity tests and risk assessment calculations. The sensitivity of different aquatic organisms to birch wood vinegar was variable and NOEC values ranged from 82 to 635 mg L^{-1}. Soil organisms were more tolerant of wood vinegar than aquatic organisms, as the NOEC for the soil dwelling earthworm *Aporrectodea caliginosa* was 2694 mg kg^{-1}. No long-term effects on soil microbes, nematodes or enchytraeids were found. The initial risk assessment indicated the risks of wood vinegar (<400 L ha^{-1}) to soil and aquatic organisms to be negligible.

Based on preliminary data, biochar reduced the leaching of glyphosate from the soil by 24–27%. The effects of wood vinegar on glyphosate leaching were inconsistent, warranting further examination. Soils treated with a mixture of biochar and wood vinegar showed the lowest glyphosate leaching, both with and without plants. Neither wood vinegar nor biochar alone had clear effects on glyphosate degradation in the soil, despite their positive influence on microbial respiration.

The studies presented in this thesis provide strong evidence for the potential of birch-derived pyrolysis liquids as an effective, non-costly and environmental friendly method against molluscs. More studies are needed to investigate the effective compounds behind the observed repellent effect. As wood vinegar is only slightly toxic or non-toxic to most non-target aquatic and soil organisms, the environmental risk due to synthetic pesticides could be diminished by including wood vinegar as part of a pest control protocol. Biochar could also play a role in pesticide risk reduction, particularly in preventing contamination of the aquatic environment. The results show, for the first time, that biochar has the potential to influence the fate of glyphosate in the soil by preventing its leaching from soil. Based on the results of this thesis research, the birch-derived slow pyrolysis liquids and biochar appear to have potential for use in sustainable plant protection. Further research is required to obtain relevant practical application technologies and to solve economical questions of their use.

ABBREVIATIONS

AMPA	Aminomethylphosphonic acid
BTO1	The aqueous phase of distillate originating during pyrolysis process, pure birch wood vinegar
BTO2	The crude viscous material generating at the end of the distillation process, wood vinegar with tar
BTOm	Mixture of BTO1 and BTO2
DW	Dry weight
EC_{50}	Half maximal (50%) effective concentration of a substance
HQ	Hazard quotient
IC_{50}	The concentration of a compound needed to reduce 50% inhibition in a specific period
IPM	Integrated Pest Management: a sustainable approach to managing pests by combining biological, cultural, physical and chemical tools in a way that minimizes economic, health, and environmental risks
K_{OC}	Soil-water partition coefficient for organic compounds
K_{OW}	The octanol-water partition coefficient: a measure of the hydrophobicity of an organic compound
LC_{50}	Median lethal concentration for 50% of test population in a specified period
NOEC	No Observed Effect Concentration: the concentration of a pollutant that will not harm the species involved
PEC	Predicted Environmental Concentration: an estimate of the expected concentration of a substance in the environment
PNEC	Predicted No-Effect Concentration: the concentration below which exposure to a substance is not expected to cause adverse effects.
REACH	Registration, Evaluation, Authorisation and Restriction of Chemical substances. European Community Regulation on chemicals and their safe use (EC 1907/2006).
TER	Toxicity-to-exposure ratio
TOC	Total organic matter

1. INTRODUCTION

1.1 Pesticides in the modern world

Pesticides are substances intended to prevent, repel, mitigate or destroy any pests that are considered to be harmful. Target pests can include insects, weeds, molluscs, mammals or microbes (FAO 2002). Presently, more than 2.5 million tons of pesticides are used each year in cultivation alone all over the world. For example, in Finland the amount of pesticides (active ingredients) sold in 2010 was 2.3 million kilograms (Savela, M., personal communication). The increased use of pesticides, especially in agriculture, and their impacts on terrestrial and aquatic environments, their biota, and functions have become a matter of considerable concern in recent decades (Stoytcheva 2011). Various pesticides are known to increase the mortality of non-target organisms, hampering the decomposition rate of organic matter and altering the physico-chemical quality of soil (Bűnemann et al. 2006, Menon et al. 2005, Sebiomo et al. 2011).

The fate of pesticides in the soil mostly depends on their persistence and adsorption properties (e.g. K_{oc}, K_{ow}), the abiotic environmental conditions (e.g. temperature, moisture, soil pH), the microbial and plant community composition and biological and chemical reactions (e.g. enzymatic transformation, photolysis, hydrolysis, oxidation, rearrangements) (Van Eerd et al. 2003). Ideally, most pesticides would be degraded over time as a result of biotic processes mediated by plants and microorganisms and by chemical reactions (Krieger and Krieger 2001). The microbial degradation of pesticides is likely to decrease when they leach below the microbiologically active plant root zone, while the chemical degradation of some pesticides may still continue in deeper soil layers (Rathore and Nollet 2012). The degradation of some pesticides can lead to the production of metabolites, which may also pose an environmental threat (Krieger and Krieger 2001). However, chemical substances applied in terrestrial ecosystems often end up in aquatic ecosystems through leaching or surface runoff (Accinelli et al. 2002, Shipitalo and Owens 2003).

Due to the various negative effects of pesticides, their use should be reduced. However, it is challenging to reduce the use of pesticides and, at the same time, fulfil the food requirements of the growing human population. In addition, global climate change is causing alterations in temperature and rainfall patterns, resulting in the ranges of crop weeds, insects and diseases expanding to higher latitudes (see reviews by Parmesan 2006, Rosenzweight et al. 2001). For example, populations of the land snail *Arianta arbustorum* and the Iberian slug *Arion lusitanicus* have increased substantially in many parts of northern Fennoscandia in recent years. As a result, these molluscs have rapidly become an increasing problem with severe impacts, both in private gardens and agriculture (Kozlowski 2007, Valovirta 2001). Consequently, due to the various negative effects of pesticides, there is an increasing need to develop new methods for pest control.

1.2 Towards more sustainable agriculture

The European Union launched "*The Thematic Strategy on the sustainable use of pesticides"* in 2006 to minimize health and environmental risks caused by the use of plant protection products. In 2009, it was accepted as a new framework directive (2009/128/EC), which fosters the development of plant protection and integrated pest management (IPM) in the EU. According to the framework directive (2009/128/EC), the use of pesticides should be reduced and replacement substances, low-risk pesticides as well as biological control measures and technologies, should be considered in the first place. The risks caused by residues and degradation products of pesticides in soils and aquatic systems should also be reduced. Plant-derived products may have a significant role in sustainable plant protection when functioning as replacement substances for synthetic pesticides (Tiilikkala et al. 2011), or alternatively by affecting the behaviour of synthetic pesticides in soil. However, even when a chemical has a natural origin, this does not guarantee that it is safer than a synthetic chemical (Ames et al. 1990). The environmental risks of such chemicals need to be evaluated and active ingredients must be authorized according to the valid regulations (Cavoski et al. 2011).

1.3 Botanical pyrolysis products as alternative pesticides

Various plant species and technologies, such as steam distillation, expression and pyrolysis, have been used in pesticide production (Chu et al. 2013, Tiilikkala et al. 2010, Tiilikkala et al. 2011). Because of divergent natural resources in different parts of the world, the raw materials used for the production of botanicals will differ. In Finland, the substantial supply of wood material has given rise to the use of birch wood (*Betula* sp.) as a basis for modern pesticides. Birch tar oil (BTO; CAS 8001-88-5 in the worldwide substance database of American Chemical Society 2007) is a crude by-product of the slow destructive distillation or pyrolysation of birch wood (including bark) for manufacturing charcoal. Pyrolysis is a thermal decomposition process in which organic compounds are transformed to gaseous, liquid and solid products in the absence of oxygen (Fengel and Wegener 1984). The chemical composition of pyrolysis products varies depending on the feedstock and pyrolysis conditions (Oasmaa et al. 2010). The suitability of birch tar oil as a biocide and/or repellent against insects, weeds and rodents has been recently tested (Salonen et al. 2008, Tiilikkala and Salonen 2008, Tiilikkala and Segerstedt 2009). Despite its potential value as a biological plant protection product, little is known about its practical pesticide value, and at the initiation of my studies I was aware of only one publication in which the applicability of birch/pine oil had been tested as a repellent against mosquitoes (Thorsell 1998).

Later on, it was discovered that the use of plant distillation/pyrolysis products referred to as wood vinegar (pyroligneous acid, mokusaku) in agriculture is an old and tradition practice in Asia (Ogawa and Okimori 2010). The pyrolysis liquid can be divided into aqueous (wood vinegar) and oil (tar) phases. In this thesis research, the two types of pyrolysis liquids were tested: BTO1, which is equivalent to wood vinegar, a water-soluble fraction resulting from the early phase of the distillation process, and BTO2, a viscous form produced at the end of the pyrolysis process and also including tar components (referred to as birch tar oil in this thesis).

During the last 10 years, the use of wood vinegar derived from various plant materials has rapidly increased and numerous botanical pesticides have come to the market in many Asian countries, but not in Europe (Tiilikkala et al. 2010). Depending on the dosage, wood vinegar can act as a biocide against microorganisms (Baimark and Niamsa 2009, Velmurugan et al. 2009), weeds and insects (Tiilikkala and Segerstedt 2009, Wititsiri 2011, Yatagai et al. 2002). When diluted sufficiently, it can be applied as soil enrichment to stimulate plant rooting, shoot growth (Wei et al. 2009) and microbial activity (Steiner et al. 2008). However, despite the long history of applying wood vinegar to soils in Asia (Ogawa and Okimori 2010), the scientific evidence for its efficacy is scarce and only a limited number of scientific publications exist focusing on pyrolysis liquids as pesticides or biocides (Tiilikkala et al. 2010). Moreover, very little is known about the toxic effects of wood vinegar in the environment (Orihashi et al. 2001, Tiilikkala et al. 2010).

1.4 Pyrolysis products as a soil amendment

Exogenous organic materials introduced to soil may have a strong effect on the degradation and adsorption behaviour of organic pesticides in the environment (Iglesias-Jimenez et al. 1997). Besides applying pyrolysis products as pesticides, these substances can also be used to improve soil quality. Recently, biochar, a form of charcoal that is added to soil (Lehmann et al. 2003), has received much interest due to its potential for improving soil fertility and plant growth. The ability of biochar to improve soil properties, plant growth and microbial activity has been extensively studied (see reviews by Verheijen et al. 2010, Lehmann and Joseph 2009). In recent years, it has also been found that biochar has the capacity to modify the environmental fate of several pesticides. Evidence suggests that biochar has a high capacity to adsorb both inorganic (Cao et al. 2009) and organic (Beesley et al. 2010, Wang et al. 2010) pollutants. Biochar appears to increase the sorption of several pesticides, such as diuron (Yu et al. 2006), simazine (Jones et al. 2011) and terbuthylazine (Wang et al. 2010). Limited degradation of pesticides (simazine, diuron) has also been observed in soils in the presence of biochar (Jones et al. 2011, Yang et al. 2006, Yu et al. 2009).

Wood vinegar has also been used in soil improvement due to its potential to stimulate plant rooting, shoot growth (Wei et al. 2009, Zulkarami et al. 2011) and soil microbial activity (Steiner et al. 2008). In Japan, it is also a common practice to apply pyrolysis-derived wood vinegar and charcoal as a mixture (called Sannekka E) to improve soil fertility (Kadota and Niimi 2004, Kang et al. 2012). Although wood vinegar could thus affect the behaviour of pesticides, for example due to the enhancement of microbial activity, knowledge of its impacts on chemical herbicides is virtually non-existent.

Glyphosate (N-(phosphonomethyl) glycine), a broad-spectrum, nonselective and post-emergence herbicide, is commonly used in agricultural and non-agricultural systems (Baylis 2000). For example, in Finland it accounted for almost 40% of herbicide-active ingredients sold in 2010 (Savela, M., personal communication). Glyphosate has unique sorption characteristics in soil when compared with other pesticides. It has a high soil adsorption coefficient (Kd = 61 g/cm^3), suggesting low mobility and thus only a marginal tendency to leach downward in the soil profile (Shuette 1999). Due to its rapid adsorption onto soil particles and vulnerability to microbial degradation, glyphosate is assumed to be rapidly inactivated immediately after spraying. This has given rise to the common belief that glyphosate is a relatively environmentally safe herbicide (Giesy et al. 2000). However, recent investigations have shown that the rate of degradation and sorption of glyphosate is dependent on soil properties (Gimsing et al. 2004a, 2004b) and climatic conditions, and the biosafety of glyphosate has been questioned (Antoniou et al. 2011, Helander et al. 2012). As a consequence, under certain environmental conditions, glyphosate and its degradation products can be prone to leaching to deeper soil layers (Borggaard and Gimsing 2008). To my knowledge, the effects of pyrolysis-derived biochar or wood vinegar on the environmental fate of glyphosate, either alone or mixed together, have not been studied.

1.5 Ecological risk assessment of natural products

1.5.1 Legislative requirements

Before the use of wood vinegars as plant protection products can become a common practice in horticultural and agricultural production in Europe, their potential as a pesticide must be scientifically proven. Furthermore, the ecotoxicological effects of wood vinegar on the environment must be assessed according to international regulations before it can be used in the field (EC 2003). Wood vinegar can be utilised as a biocide, a plant protection product or in various other products such as paints, compost odour removers and medicines (Tiilikkala et al. 2010).

To apply wood vinegar as an approved product in EU markets, it should be approved as an active substance according the various statutes, depending on the use. When used as plant protection product (in Finland) the approval process should be carried out according to the Finnish Plant Protection Products Act (1563/2011) and the Plant Protection Products Regulation (1107/2009) by European Union. However, when applied as a biocide the approval must meet the requirements set by the Biocidal Products Directive 98/8/EC (BPD). In addition, when used in other products, wood vinegar should be registered according to Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH). Only when the active substances are included in the Annexes of the abovementioned statutes, they can be used in biocidal, plant protection, or other products targeted at the EU market.

It would be reasonable to start to implement wood vinegar as part of the REACH registration process that encompasses about 80% of those information requirements also required in 1107/2009 and 98/8/EC. For substances produced or imported in quantities over 10 tons per producer or importer per year, REACH registration insists on risk assessment, which includes identifying the hazards, exposure information on the chemicals and, based on this, the analysis of risks to human health and the environment (Backhaus et al. 2010). The data must be of good quality to produce a scientifically valid risk assessment of impacts on non-target species. The data presented in this thesis are intended to fulfil such criteria.

1.5.2 Current EU risk assessment practices

Assessment of the risks of chemicals to the environment is a complex task (SANCO 2002). General risk assessment includes four phases: hazard identification, exposure assessment, dose-response assessment and risk characterization (Newman and Unger 2002). Current EU risk assessment practices are mostly based on estimating the toxicity of single chemicals (EC 2010). Risk assessment of mixtures can be grouped into component-based approaches (CBAs) and direct toxicity assessments (DTAs), depending on the aims of the risk assessment protocol. Consequently, an important factor in assessing risks for mixtures is the availability, or absence, of reliable data that include the identity, toxicokinetics, metabolic pathways, mechanisms of action and levels of exposure for the whole mixture or its separate components (IGHRC 2009). In ecological risk assessment the aim is usually at the protection of populations rather than individuals. The continuance of populations of non-target organisms should be ensured (SANCO 2002).

When a mixture of chemicals is an outcome of a particular process, such as pyrolysis, and/or from various sets of parent materials, it is not clear which compounds act as active substances and which are non-acting impurities. It is also possible that such mixtures do not have a well-defined chemical composition (IGHRC 2009), which further complicates the registration of botanicals as pesticides. Mixtures whose chemical composition cannot be completely identified are generally treated as single substances under REACH (EC 2010).

In a mixture, compounds can interact with each other and the joint effect can be additive, synergistic or antagonistic (Newman and Unger 2002). Consequently, if a mixture is composed of hundreds of chemicals, it is reasonable to perform risk assessment using data on the entire mixture to determine the actual effects of the mixture in the environment. In environmental risk assessment the volumes of tested chemicals in various uses should be investigated properly. Usually, when assessing the ecological risks of a mixture, exposure assessment is the area on which most emphasis should be placed (SCHER et al. 2012). Birch wood vinegar consists of hundreds of compounds (Fagernäs et al. 2012 a, b). As each individual compound has specific physicochemical and (eco)toxicological properties, and potential for being moved and degraded in different parts of environment, determination of the potential environmental risks of birch wood vinegar is challenging.

1.5.3 Deriving risk assessment data in the terrestrial environment

Predicted environmental concentration (PEC) is used as an estimate of the expected concentration of a substance in the environment (EC 2003). The predicted no-effect concentration (PNEC) is used to represent the concentration below which exposure to a substance is not expected to cause adverse effects. To calculate the PNEC, an assessment factor (1, 10 or 100) is applied to the lowest available toxicity value (NOEC or $L(E)C_{50}$) (EC 2003). A low extrapolation factor can only be used when one has a large and validated data set (EC 2003).

In terrestrial risk assessment quotients are commonly applied to combine exposure and effect in order to characterise the risk (SANCO 2002). There are many ways in which such quotients can be formally derived. Currently it is used for toxicity-to-exposure ratios (TER) along with hazard quotient (HQ) values. The TER value is a comparison between an estimate of an ecological effect on the most sensitive species (e.g., LD_{50}, LC_{50}) and of the estimated exposure in the realistic worst case. The TER value should be used as an indicator of risk in the assessment process (EC 2003). The ecological risk of a substance in the environment can also be estimated numerically using the hazard quotient (HQ) approach. The HQ is the ratio of the exposure estimate to an effect concentration considered to represent a "safe" environmental concentration (SANCO 2002). Deriving these values to wood vinegar to estimate its risk on environment is essential when the aim is to use it in plant protection or other uses in EU.

2. OBJECTIVES OF THE PRESENT STUDY

The aim of this thesis research was to explore the efficacy of birch-derived slow pyrolysis liquids, birch tar oil (BTO2) and wood vinegar (BTO1), in mollusc control and to investigate the environmental risks of their use. In addition, the effects of birch-derived biochar and wood vinegar on the environmental fate of glyphosate were investigated.

The study reported in the first paper (I) examined the potential of pyrolysis liquids as pesticides against two molluscan species, the Iberian slug *Arion lusitanicus* Mabille (Gastropoda: Arionidae) and the land snail *Arianta arbustorum* L. (Gastropoda: Helicidae). It was examined whether birch tar oil or wood vinegar, could be applied as a plant protection product for the control of land snails by direct topical spray application. In addition, it was investigated whether birch tar oil used either alone, mixed with wood vinegar or with Vaseline® could be usedas a repellent against slugs and snails when painted on a fence.

According to international regulations, the ecotoxicological effects of chemicals on the environment must be assessed before their use in the field (EC 2003). The ecotoxicological effects of wood vinegar and birch tar oil on non-target soil organisms were monitored via changes in soil fauna and plant populations in the field, and in greenhouse and laboratory experiments (II, IV). Three groups of soil organisms covering various trophic levels were monitored: enchytraeid worms (mostly omnivorous), nematodes (covering several trophic positions) and soil microbes (primary decomposers). These soil organisms were selected to test the effects of wood vinegar and birch tar oil on non-target soil biota, because they fulfil several criteria required for toxicity tests. These biota are present in a wide range of ecosystems, occur abundantly, play a key role in the functioning of the soil ecosystem, are easy to use, collect and culture, come into contact with a variety of stress factors (the soil solution, the solid phase and the gaseous phase in soil) and are sensitive to environmental stresses (Didden and Römbke 2001, Römbke and Moser 2002, Schloter et al. 2003). In addition, the median lethal concentration (LC_{50}) and the no observed effect concentration (NOEC) were determined for a soil-dwelling organism, the earthworm *Aporrectodea caliginosa*, and the median effective concentration (EC_{50}) and the NOEC for the reproduction of the springtail (Collembola), *Folsomia candida* (II).

The acute toxicity of birch wood vinegar (EC_{50} values, i.e. the concentration of wood vinegar producing a certain half-maximal effect) was assessed on an extensive group of aquatic organisms widely used in ecotoxicological studies (III). Of the aquatic organisms used, the water louse *Asellus aquaticus* and the oligochaete worm *Lumbriculus variegates* are sediment-dwelling benthic invertebrates, while the pond snail *Lymnaea* sp. usually lives on aquatic plants. The pelagic and littoral organisms were represented by the water flea (*Daphnia magna*), lesser duckweed (*Lemna minor*), zebrafish (*Danio rerio*), unicellular green algae (*Scenedesmus gracilis*) and fluorescent bacteria (*Vibrio fischeri*).

When mixed in arable soil, wood vinegar increases the soil microbial activity (III) Steiner et al. 2008). Biochar has also been shown to enhance soil microbial activity (Lehmann et al. 2011). However, unlike wood vinegar, biochar is additionally an active sorbent (Beesley et al. 2010, Wang et al. 2010). In the fourth paper (IV), the ability of wood vinegar and biochar to reduce glyphosate-induced soil and water pollution by stimulating the activity of glyphosate-degrading microbes was examined.

The main objectives of this thesis were to address the following questions: (i) Does birch tar oil alone or mixed with wood vinegar have potential application value in controlling slugs and snails? (ii) Does birch tar oil and wood vinegar application cause a risk to non-target terrestrial and aquatic organisms? (iii) Do wood vinegar and biochar affect the environmental fate of glyphosate? Furthermore, this introduction chapter aims at taking one step further, i.e. placing my observations into a wider environmental context. The objective is thus to apply the results of the separate publications in drawing comprehensive conclusions concerning the potential of slow pyrolysis products, especially pyrolysis liquids, in sustainable plant protection and to identify the environmental risks of their use. The perspective of this thesis is mostly in the ecological point of view. Economical and commercial questions are not concerned. Toxicity values achieved in the separate studies (II, III) are used as a basis of the tentative environmental risk assessment of wood vinegar. The estimated environmental exposure is compared to the estimated effects according to the current EU documents for risk assessment. The probability of the wood vinegar to cause an environmental risk is judged numerically basing on the predicted environmental concentration (PEC), predicted no effect concentration (PNEC), toxicity-to-exposure ratios (TER) and hazard quotient (HQ) values (EC 2003).

The main hypotheses were:

1) Birch tar oil and wood vinegar eliminates and repels slugs and snails (I).
2) Wood vinegar and birch tar oil are non-toxic or only slightly toxic to non-target soil and aquatic organisms (II, III).
3) As an active sorbent, biochar reduces the leaching loss and decreases the degradation of glyphosate in soil (IV).
4) Wood vinegar increases the degradation of glyphosate by stimulating soil microbial activity (IV).
5) Derived risk assessment values (TER, HQ) of wood vinegar indicates no risk on soil non-target fauna.

3. MATERIALS AND METHODS

3.1 Materials

Pyrolysis liquids of two kinds, both derived from pyrolysed birch (*Betula pendula*) wood and bark, were supplied by Charcoal Finland Ltd. The first type (BTO1), wood vinegar, consists of the water-soluble fraction resulting from the early phases of the distillation process, i.e. at temperatures less than 380°C. The second type (BTO2) is a viscous form of birch tar oil produced at the end of the pyrolysis process when the temperature reaches 400°C (I, II, III). The pyrolysis liquids arising during the distillation process were first collected in a large tank, after which the lighter part (wood vinegar) was separated from the heavier "tar oil" by decanting. The wood vinegar used was a crude fraction also containing some soluble tar. It had an organic matter content of about 57% and pH 3.1. In study IV, a purer form of wood vinegar was applied, which was derived from bark-free heartwood birch material from a plywood mill and supplied by Raussi Energy Ltd. (Finland). This "pure" wood vinegar contained no tar and its organic matter content varied between 25 and 30% (Table 1).

The biochar was derived from birch wood (including bark) and pyrolysed by Tisle Suomi Ltd. at 450 °C for a holding time of 23 h. To obtain information on the greatest possible risks and benefits of the three substances, relatively high concentrations of wood vinegar and biochar were used in the experiments (I–IV). The complete pyrolysis processes, the composition and analyses of the used birch tar oil, wood vinegar and biochar are reported in Fagernäs et al. (2012a, b). In their article Fagernäs et al. (2012 a, b) marked different pyrolysis retorts by letters A, B and C. In my Papers I, II and II the used birch tar oil and wood vinegar are the products of retort C. Samples for the chemical analyses of the liquids used in papers I, II and III were taken from a different batch than used in exams. As the retort, pyrolysis process and feedstock material were constant, the composition of used liquids is assumed to be similar between batches. In the Paper IV, the used wood vinegar is from retort A while the biochar from retort B.

Table 1. Characteristics of the wood vinegars and birch tar oil used in different Papers.

	pH	**Organic matter, wt %**	**PAH mg kg^{-1}**	**Study**
Wood vinegar	3.1	57.0	290	I, II, III
Wood vinegar	2.0	25.3	21	IV
Birch tar oil	2.8	86.8	2000	I, II

3.2 Experiments with snails and slugs

3.2.1 Direct spray application

A laboratory experiment was performed in 1.7 L glass jars with a soil monolith (3 cm thick) growing plants in 2003 (I). Four mature snails and 55–65 eggs were placed on the soil in each jar. Three treatments each with 5 replicates, were established: jars sprayed once with 1) birch wood vinegar (500 L ha^{-1}), 2) birch tar oil (500 L ha^{-1}) and 3) an equal amount of water (control). Hatching of the eggs and movement of the adult snails were observed weekly. After three months, the snails were removed to clean jars with fresh plant material to activate them and to check their survival. The following day, the number of surviving snails was recorded.

3.2.2 Repellent effect of birch tar oil on *A. arbustorum*

To study the degree to which birch tar oil repels *A. arbustorum*, two experiments were conducted with birch tar oil (I). In the first experiment (1), plastic fences (height 40 cm, covering an area of 0.74 m^2, partly buried in the soil) were established in five private yards with grassy vegetation in the city of Lahti in 2005. The fences received four treatments, each with three to five replicates: 1) fences without Vaseline® and birch tar oil; 2) birch tar oil smeared on the fences; 3) fences receiving Vaseline® only; and 4) fences with a mixture of Vaseline® and birch tar oil. The substances were spread using a brush on the inner upper side of the fences to form a 10 cm-wide barrier. The upper 5 cm of the fence was bent to form a “rain shadow” covering the area on which the repellent was applied. Treatments were applied to the fences only once at the start of the study. The next day, 50 snails were placed in each fenced area. The study lasted for 38 days, and within this period, the number of snails in the fenced areas was monitored 10 times.

In the second experiment (2), the setup was identical to that in experiment (1) except that no snails were added to the fenced areas. The snails present inside the fences were removed prior to starting the experiment. The experiment was conducted in the city of Lahti in a fertile fallow meadow growing tall herbs, grasses and some deciduous trees in 2005. The *A. arbustorum* population in the meadow was >10 adults m^{-2}. Pieces of carrot were placed inside the fence to attract snails into the fenced area. The study lasted for 42 days, within which time the entrance of snails into the fenced area was monitored five times.

3.2.3 Repellent effect of birch tar oil and wood vinegar as a mixture on *A. lusitanicus*

An experiment was established at MTT Agrifood Research Finland, Jokioinen, in 2005 (I). The slug population in the experimental field varied from a few individuals to 20–100 individuals m^{-2}. The experiment consisted of 24 pots growing Chinese cabbage seedlings. A mixture (BTOm) of birch wood vinegar and birch tar oil (30/70, v/v) was painted on the whole outer surface of the pots either weekly or fortnightly, while control pots received no treatments (n = 8). Half of the pots were equipped with a plastic collar, 3 cm in breadth, fastened around the rim of the pots to prevent them being washed by raindrops. The plants were checked in the morning on a daily basis for the duration of the study. The number of slugs entering the pots and accessing the plants was counted. The damage to the plants caused by the slugs was estimated by visual assessment as the percentage of the damaged leaf area. Observations were continued until it could be verified that slugs had entered all treatments.

3.3 Effects of birch tar oil, wood vinegar and biochar on soil organisms and plants

The ecotoxicological effects of wood vinegar and birch tar oil on soil organisms were monitored via changes in soil fauna and plant populations using garden, field and mesocosm experiments (II) and in a greenhouse experiment (IV). Three groups of organisms of various trophic levels were chosen: enchytraeid worms (mostly omnivorous), nematodes (covering several trophic positions) and soil microbes (primary decomposers). To estimate the highest possible risks of the substances, relatively high doses were used in the experiments.

In the “garden experiment”, six 2 m^2 plots, enclosed by fences, were constructed in June 2003 in five private gardens the city of Lahti (II). Three treatments were established in each garden with two replicates: the plots were sprayed with 1) birch wood vinegar (500 L ha^{-1}), 2) birch tar oil (500 L ha^{-1}) and 3) tap water (= control) (n = 10 for each treatment). Two litterbags (mesh size of 1 mm) containing 2 g (dry mass) of *Calamagrostis arundinacea* (Poaceae) leaf litter were placed in the soil to a depth of ca. 1 cm in each plot to examine the effects of the tested pyrolysis substances on the decomposition rate of litter (n = 10). Two soil samples were taken from each plot four times during the study (70 d), and numbers of nematodes and enchytraeids were counted (for methods see below). The effect of birch tar oil and wood vinegar on plants (total plant coverage %) was estimated concurrently with the taking of soil samples. At the final sampling, plants were harvested from randomly selected 50 × 50 cm^2 areas in each plot, identified, dried and weighed.

The “field experiment” (II) was carried out in an experimental field in central Finland, Toholampi, in summer 2005. An arable field containing numerous weed species was divided into ten contiguous plots (1 × 2 m). Five randomly chosen plots were sprayed with wood vinegar (1360 L ha^{-1}) once at the start of the study using a compressed air pump. The control plots (n = 5) were treated with water only. The experiment was conducted over 42 days, within which time soil samples were taken five times. At each sampling time, three soil samples were taken from each plot for the analysis of the numbers of nematodes, the biomass of enchytraeids, and the activity and biomass of soil microbes.

The mesocosm studies were established in a garden area in Lahti in summer 2004 (II). Experiment 1 consisted of 75 mesocosms established in 1500-mL glass jars filled with 400 g of fresh homogenised garden soil. Grass (*Festuca rubra*, *Festuca ovina* and *Poa pratensis*) seeds were sown in the mesocosms and kept under a plastic cover in natural light and temperature conditions in the garden. After a stabilization period of one month, three treatments were established, each with five replicates: the mesocosms were treated once with: 1) 100% wood vinegar (500 L ha^{-1}), 2) 5% wood vinegar (500 L ha^{-1}) or 3) water (= control). Five jars per treatment were randomly selected on days 1, 7, 20, 29 and 48 for destructive sampling, in which the effects of the treatments on the numbers of nematodes, biomass of enchytraeids and microbial activity were examined. After the last sampling, the plants were uprooted, dried and weighed. Mesocosm experiment 2 was identical to experiment 1 described above, except that the former was conducted in 200-mL plastic jars containing 100 g garden soil, and no plants were sown in the mesocosms. The mesocosms were kept at room temperature (+ 22 ^{o}C) in constant darkness.

The impacts of wood vinegar and also biochar on soil organisms and plants were investigated in the greenhouse at MTT Agrifood Research Finland, Jokioinen, in summer 2010 (IV). The “greenhouse experiment” was conducted in

1500-mL flowerpots. Four treatments, each with 20 replicates, were established: all treatments included arable soil mixed with 1) biochar (51 t ha^{-1}), 2) wood vinegar (2000 L ha^{-1}), 3) biochar and wood vinegar (51 t ha^{-1}, 2000 L ha^{-1}), or 4) a control system with neither biochar nor wood vinegar additions. The experimental and sampling design is described entirely in section 3.5.

Nematodes were extracted from 5 g (fresh) soil samples using the wet funnel method by Sohlenius (1979), and enchytraeids were extracted from samples of 30–80 g soil using the wet funnel technique described by O'Connor (1955). Microbial activity was measured using basal respiration as an estimate. The microbial biomass was determined using the substrate-induced respiration (SIR) method described by Anderson and Domsch (1978).

3.4 Aquatic toxicity tests

The acute toxicity (LC50/EC50) of birch wood vinegar to an extensive group of aquatic organisms widely used in ecotoxicological studies was investigated (III). Bioassays with *Asellus aquaticus* (crustacean), *Lumbriculus variegatus* (oligochaete worm), *Daphnia magna* (crustacean), *Lymnaea sp*. (mollusc), *Lemna minor* (vascular plant), *Danio rerio* (fish), *Scenedesmus gracilis* (algae), and *Vibrio fischeri* (bacterium) were performed according to ISO, OECD or USEPA guidelines (III). At least five exposure concentrations were applied in a geometric series without adjusting the pH after birch wood vinegar application. The test organisms were added to test jars (n = 3–5 per treatment) and the following response variables were determined: 1) root length and leaf number of duckweed (IC_{50}, *L. minor*), 2) mobility of the water flea (EC_{50}, *D. magna*), 3) survival rate of the water louse (LC_{50}, *A. aquaticus*), zebrafish (LC_{50}, *D. rerio*), oligochaete worm (LC_{50}, *L. variegatus*) and pond snail (LC_{50}, *Lymnaea sp*.), 4) inhibition of the light emission capacity of bacteria (IC_{50}, *V. fisheri*) and 5) the number of cells of the alga *S. gracilis*. The organisms used in the short-term toxicity test were not fed during the tests.

Soil-dwelling earthworms and springtails are widely used in laboratory toxicity tests because of their important roles in ecosystems and sensitivity to numerous chemical stressors. The grey worm *Aporrectodea caliginosa* is a dominant endogeic earthworm species in the agro-ecosystems in Northern Europe (Kula and Larink 1998, Nieminen et al. 2011). The collembolan *Folsomia candida* is among the most sensitive springtails to an array of chemicals (Chernova et al. 1995). To test the toxicity of wood vinegar on soil organisms, we determined the LC_{50} and NOEC values of birch wood vinegar to *A. caliginosa* and the EC_{50} and NOEC values for the offspring production of *F. candida* according to OECD and ISO guidelines (II).

3.5 Effects of birch wood vinegar and biochar on the degradation and leaching of glyphosate

The impacts of wood vinegar and biochar on the degradation and leaching of glyphosate were investigated in a greenhouse at MTT Agrifood Research Finland, Jokioinen, in summer 2010 (IV). The experiment was conducted in 1500-mL flowerpots. The four treatments, each with 20 replicates, consisted of soil mixed with 1) biochar, 2) wood vinegar, 3) biochar and wood vinegar, or 4) a control system with neither biochar nor wood vinegar additions. The application rate of biochar in the pots corresponded to 51 t ha^{-1}, assuming a 10-cm incorporation depth (3.3% biochar content by dry mass). The wood vinegar concentration applied in the pots corresponded to 2000 L ha^{-1} (0.26%). The experiment ran for 82 days, within which time soil and water leachate samples were taken three times (on days 4–5, 46–

47 and 80–81). After the first sampling, seeds of English rye grass (*Lolium perenne*) were sown in half of the pots to determine the effects of plants on the fate of glyphosate. When the grass reached a height of ca. 20 cm (day 36), half of the pots (with and without plants) were treated with glyphosate (Roundup Bio; Monsanto, Copenhagen, Denmark) mixed with water (1:100) corresponding to 2000 mL active ingredient ha^{-1}. Four days after the addition of glyphosate, a second addition of wood vinegar (500 L ha^{-1}) was made for pots that already contained wood vinegar. This was done to ensure that enough wood vinegar was present in the soil to stimulate glyphosate degradation by soil microbes.

At each sampling, two soil samples were taken from each pot using a corer and stored at 5 °C for the analysis of microbial activity and counting of nematodes. Soil samples for analysis of glyphosate and its degradation product, AMPA, were taken 44 days after glyphosate addition. One day after each soil sampling event, pots were irrigated with 300 mL of tap water to mimic heavy rain. The water leaching through the soils was quantified and collected for analysis. After measuring the conductivity, pH and TOC of each leachate sample, the leachates were pooled within a treatment to obtain one composite sample per treatment to analyse the concentration of glyphosate, AMPA, and the components of wood vinegar. The acute toxicity of the leachates was investigated using the *D. magna* Acute Immobilisation Test 202 (OECD 2004) with minor modifications. At the end of the study, the plants were uprooted, weighed and dried. The shoot and root biomass of the plants were determined separately.

Statistical analyses were performed using conventional tests such as ANOVA. The statistical software package SPSSS c.15 for Windows was applied (SPSS 1999).

3.6 Deriving formal risk assessment data for wood vinegar

3.6.1 Risk assessment of wood vinegar as a mixture

As a part of the initial risk assessment the potential use targets and volumes of wood vinegar in various uses were investigated. The predicted environmental concentration PEC (mg kg^{-1}) of wood vinegar in the soil immediately following a single application was calculated according the following formula (FOCUS 2006):

$$PECsoil = \frac{A \times (1 - fint)}{(100 \times depth\ \times bd)}$$

A = application rate (g ha^{-1})
fint = fraction intercepted by crop canopy
depth = mixing depth (cm)
bd = dry soil bulk density (g cm $^{-3}$)

In initial risk assessment, the estimated exposure is compared to the estimated effects. The initial risk characterization of wood vinegar was performed by means of toxicity-to-exposure ratios (TER) ja hazard quotient (HQ) values. The TER value is a comparison between an estimate of an ecological effect on the most sensitive species (e.g., LD$_{50}$, LC$_{50}$, NOEC) and of the estimated exposure in the realistic worst case (EC 2003). The TER value for wood vinegar was calculated according the following formula (SANCO 2002):

$$TER\ = \frac{LC\,50\ (mg/kg)}{\begin{array}{c} realistic\ worst\ case\ PECs \\ (initial\ or\ short\ term\ in\ mg/kg) \end{array}}$$

In the *Council Directive* concerning the marketing of plant protection products (91/414/EEC, Annex VI), boundary values are presented for the TER to account for uncertainties (e.g. lab to field or tested species vs. all species). Annex VI (91/414/EEC) specifies the decision rule:

TER ≥ 10 for acute risks and ≥ 5 for long-term risks.

As a part of the exposure scenarios, predicted no-effect concentration (PNEC) values of wood vinegar were determined for freshwater and soil organisms based on available toxicological information (II, III) and according to current REACH guidance documents for hazard assessment. The $\mathrm{PNEC}_{\mathrm{aqua(freshwater)}}$ and $\mathrm{PNEC}_{\mathrm{soil}}$ values were calculated according the NOEC values of the most sensitive aquatic and soil organisms (II, III) to birch wood vinegar and using correction factors 10 and 100.

The ecological risk of wood vinegar in the environment was also estimated using the hazard quotient (HQ) approach. The HQ is the ratio of the exposure estimate to an effect concentration considered to represent a "safe" environmental concentration (SANCO 2002). In environmental risk assessment, this is based on the ratio of the predicted environmental concentration (PEC) and predicted no effect concentration (PNEC) (EC 2003). HQ value for wood vinegar was assessed according to the following formula (SANCO 2002):

$$HQ = \frac{\begin{array}{c} maximum\ environmental \\ concentration\ or\ the \\ calculated\ dose\ estimate \end{array}}{screening\ ecotoxicity\ value}$$

HQ values less than 1.0 are considered to indicate an acceptable risk, whereas HQ $\geq$ 1.0 indicates an unacceptable risk. If the HQ ratio of 2 for arthropods is exceeded, a litter test is required (Mattsoff 2005). The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC concludes that if the off-field HQ (where the correction factor of 10 has been applied) is less than 2, no further assessment is required (SANCO 2002).

3.6.2 Compound-specific exposure assessment of wood vinegar

The five most abundant components of birch-derived wood vinegar are: acetic acid, methanol, 1-hydroxy-2-propanone, acetone and furfural (Fagernäs et al. 2012b). In this part of study, an brief assessment was performed of the environmental risk from these five abundant compounds of wood vinegar by comparing their predicted environmental concentration in the soil after wood vinegar addition to the $\mathrm{PNEC}_{\mathrm{soil}}$ values found from the literature to calculate PEC/PNEC ratios. If the PEC exceeds the PNEC, i.e. the ratio is more than one, there is considered to be a risk of environmental damage (EC 2003), and further risk characterization was done by investigating the behaviour of chemicals in the environment basing on their chemical properties. A ratio of less than one indicates a low environmental risk (EC 2003).

4. RESULTS AND DISCUSSION

4.1 Efficacy of birch tar oil and wood vinegar in mollusc control

4.1.1 *A. arbustorum*: direct spray application

Contrary to our hypothesis, birch tar oil (BTO2) and wood vinegar (BTO1) proved to be ineffective in eliminating snails; neither of the substances had a statistically significant effect on the number of hatched eggs or the survival of adult and young snails in the laboratory microcosms (I). After spraying, the adult snails in the treated jars were inactive and secreted a slime plug in the front aperture of the shell. During the 3-month study period, most adult snails in the systems treated with birch wood vinegar and birch tar oil remained passive, while those in the control treatments were active. After being transferred to jars with fresh food at the end of the study, almost all adults, irrespective of the treatment, were still alive (I).

Preliminary studies (B. Lindqvist et al. unpublished data) have clearly shown the negative influence of 100% wood vinegar on the two slug species *Deroceras agreste* and *Arianta lusitanicus*: wood vinegar sprayed over land areas growing grasses and herbs resulted in the death of these molluscs soon after spraying. However, in the present study, the mortality effect of birch tar oil and wood vinegar on snails, irrespective of their age, was low. This suggests that the shells of snails provide these organisms an efficient shelter against substances that are seemingly toxic to other molluscs. The slime plug secreted by the snails in the frontal aperture further enhances their survival under unfavourable, even hostile conditions.

That the adult snails became temporarily inactive for a period of three months after the birch tar oil and wood vinegar treatments indicates that the food source of the snails, also receiving spray, remained repellent for a long time. Thus, birch tar oil and wood vinegar could still be useful in IPM strategies, where the aim is not to kill pests, but rather to prevent yield losses. The observations in the current study imply that yield losses could be reduced by the long-lasting inactivating effect of these substances on snails, thereby reducing the damage caused by snails in northern latitudes where the growing season is a short. Furthermore, a relatively long period of inactivity is certain to affect the fecundity and fertility of *A. arbustorum*, which is likely to have a negative impact on the population densities of the snails. It should be noted that the inactivating effect of birch tar oil and wood vinegar on snails in the field would be shorter, as the effect of active substances is likely to be reduced by rain and UV light.

4.1.2 Repellent effect of pyrolysis liquids on slugs and snails

As hypothesised, birch tar oil and the mixture of birch tar oil and wood vinegar exhibited a clear repellent effect against *A. arbustorum* and *A. lusitanicus* when applied as a painted barrier on the sides of a fence in outdoor conditions heavily infested with these molluscs (I). The day after placing the snails in the fenced systems, only 20% remained in the control systems, while all individuals were still present in the systems painted with a mixture of Vaseline® and birch tar oil (I). The results of experiment 2 support those obtained from experiment 1, i.e. the repellent effect was most persistent when birch tar oil was mixed with Vaseline®: none of the snails crossed the birch tar oil+Vaseline® barrier of the fenced systems during the 43-day experiment. Both birch tar oil and Vaseline® alone repelled the snails to some extent, but these effects were short term and less effective when compared to the results produced by the Vaseline®+birch tar oil mixture (I).

It was found that mixture of birch tar oil and wood vinegar (BTOm) effectively repelled *A. lusitanicus* from potted cabbage plants when applied as a protective barrier around the plastic pots (I). The plants in the control pots were completely consumed 18 days after start of the experiment, but plants in BTOm-painted pots were left almost untouched (I). There was no difference in the repelling effect between the weekly and fortnightly applications. Repeated applications to the cabbage pots over a period of several weeks were required to maintain the repellent mode of action against *A. lusitanicus*. In doing so, the concentration of the active constituents was maintained at a level high enough to prevent slugs from crossing the BTOm barrier. Weekly treatments with BTOm provided the best protection against slugs, as it took them more than three weeks after the last treatment to enter the pots. Moreover, the interval between the treatments should preferably not exceed two weeks, which seems to be the critical point for the BTOm barrier to start breaking down.

According to the results in experiments with *A. arbustorum*, it can be assumed that birch tar oil mixed with a greasy substrate such as Vaseline® could also extend the repellent effect against *A. lusitanicus*. Although the mechanism is not yet known, Vaseline® possibly prevents birch tar oil from drying, thereby retaining the repelling volatiles in the mixture. Vaseline® could also prevent the water-soluble compounds from dissolving and leaching out under heavy rain.

Interestingly, there appears to be group of compounds in the birch tar oil and wood vinegar that acts as an efficient repellent against both slugs and snails. The molluscs appear able to detect the repellent compounds in these substances by olfaction from only a short distance. When confronted with birch tar oil or wood vinegar, the molluscs stop at a distance of approximately 1 cm from the substrate, and turn around to escape from the obviously unpleasant odour (Hagner 2005, Pasanen 2006). Notably, common pine tar (with a manufacturing process having similarities to that of birch tar oil) has a similar physical structure and odour to birch tar oil (and wood vinegar), but is far less effective at repelling molluscs. Where pine tar is concerned, the snails stop by the substrate for a while but then glide over the sticky substrate with slightly increased mucus production (Hagner 2005).

The locomotion of slugs via olfactory cues is a well-know phenomenon (Gelperin 1974). Some plant extracts, such as extracts of *Saponaria officinalis* and *Valerianella locusta*, are known to have a similar effect on the behaviour of *A. lusitanicus* (Barone and Frank 1999). Further studies are needed to determine how many treatment repetitions or which concentrations give the best protective result against molluscs. As the repellent studies with molluscs were carried out with birch tar oil or a mixture of birth tar oil and wood vinegar, further studies are needed to determine whether wood vinegar, when applied alone, has similar repellent effect on slugs and snails as birch tar oil (I).

To my knowledge, the use of raw birch tar oil in plant protection is not probable as the polycyclic aromatic hydrocarbons (PAHs) are usually concentrated in the tar fraction of pyrolysis liquids (Fagernäs et al. 2012a). Instead, wood vinegar is easier to utilize and commercialize in practical use and has thus a good potential to be used for example as biodegradable pesticides. The tar and PAH contents of slow pyrolysis-derived wood vinegar are low (or could be lowered easily) and should not prevent their utilization (Fagernäs et al. 2012a, b).

In our further studies (M. Hagner et al. 2011, unpublished) we examined the repellent effect of birch wood vinegar (without the heavier tar compound) against snails. The chemical composition of birch wood vinegar and birch tar oil were analysed (Fagernäs et al. 2012b) and the repellent effect of wood vinegar and its various fractions on snails was investigated in a laboratory study. Cardboard circles were dipped (5 min) into tested wood vinegar solutions or its separate fractions for five minutes (n=7). After that the circles were placed on a moist burlap and three snails were placed inside each circle. Escaping time of snails from the circles were calculated. During the study, none of the snails crossed the wood vinegar-treated boundaries (Fig. 1). Of the tested substances, acetic acid and furfural were the most effective repellents against snails (Fig. 1). This new finding indicates that the repellent effect is not explained by one specific compound in wood vinegar but a group of compounds. The repellent effect seems not to depend on the tar fraction, as pure wood vinegar without tar is sufficient to efficiently repel snails (Fig. 1). However, wood vinegar contains high concentration of acetic acid and furfural (Fagernäs et al. 2012 b), which have been

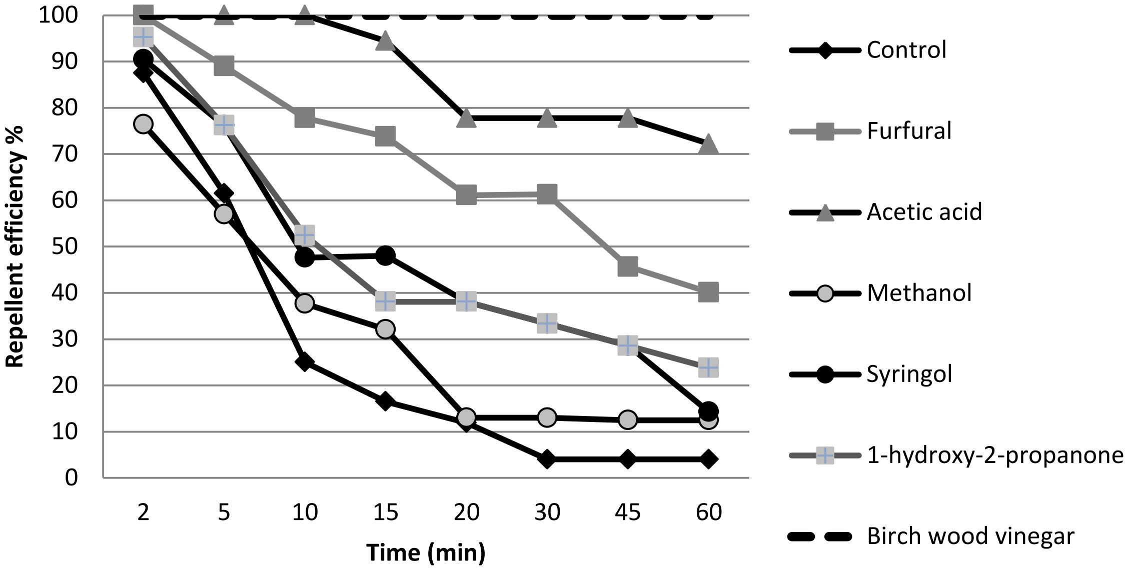

Figure 1. Repellent effect of wood vinegar on snails in relation to its main compounds (33% dilution) (M. Hagner et al. unpublished).

used as pesticides for a long time (Hensley and Burger 2006, Abouziena et al. 2009, Ismail and Mohammed 2007) .

To conclude, these studies provide strong evidence for the potential of pyrolysis liquids to be applied as an effective, non-costly, easy-to-use and environmentally friendly method against molluscs. As biological plant protection methods are needed to replace potentially harmful chemical molluscicides, pyrolysis liquids could be applied as a part of an alternative pest management strategy, not only in private gardens, but also to some extent in organic farming practices and IPM strategies. In addition to snails and slugs, recent studies have demonstrated that wood vinegar also repels other species such as psyllids (*Trioza apicalis*) and acts as a fungicide and insecticide, for example against aphids (Tiilikkala and Segerstedt 2009, Tiilikkala et al. 2011). However, application technologies and the final product may need to be refined to produce a more user-friendly form, as the sticky birch tar oil and wood vinegar blocks the pumps of spray applicators and dirties the clothes.

4.2 Effects of birch tar oil, wood vinegar and biochar on non-target soil organisms

Birch tar oil and wood vinegar had no consistent effects on enchytraeid worms either in the garden soil in the city of Lahti or in the Toholampi field study (II). Neither did the numbers of nematodes differ significantly between the control soils and those treated with birch wood vinegar or birch tar oil in the garden experiment, the mesocosm experiments or the greenhouse experiment. In the Toholampi field study, the number of nematodes in birch wood vinegar-treated plots decreased at the last sampling time when compared to the control soils. This could have resulted from the withering of the plants in the wood vinegar-treated plots, also leading to a decreased amount of root exudates from the dead/wilting plants, which can drastically reduce the nutritional resources (Martikainen 2003). However, this negative effect is likely to be short term due to the resource input in the form of dead plant biomass later on in the growing season (II).

Wood vinegar had no effect on microbial activity in the mesocosms with plants or in the greenhouse experiment (II, IV). However, in the absence of plants, microbial respiration in the mesocosms treated with birch wood vinegar increased 1 day after application, being significantly higher than in the control systems. Similarly to the mesocosm experiment without plants, microbial activity was positively affected soon after the addition of wood vinegar in the field experiment in Toholampi. This is a typical reaction when added resources are rapidly consumed by microbes (Meli et al. 2003). A list of substances found in wood distillates is given by Fagernäs et al. (2012b): typically, wood vinegar is high in low-molecular weight acids (formic and acetic), alcohols (methanol) and aldehydes, which can serve as a carbon and energy resource for prototrophic bacteria occurring in the soil (Focht 1999).

Blin et al. (2007) focused at the biodegradation of pyrolysis oils in their study. They showed water soluble part of slow pyrolysis liquid (made from spruce) to reach 62% biodegradation during 30 days being more easily mineralized by the bacteria and fungi than fast pyrolysis oils. According OECD protocol, to be classified as readily biodegradable, a compound must achieve 60% degradation in 28 days from which fist 10% should be reacted during 10 days (EC 1992). Aquatic slow pyrolysis liquid (which corresponds to wood vinegar) meets these criteria and could thus be classified as readily biodegradable (Blin et al. 2007). However, in the Toholampi field experiment, from day 9 onwards, the influence of birch wood vinegar on microbial activity was negative, but instead, wood vinegar did not reduce microbial biomass. This reduction in microbial activity, coinciding with the withering plant biomass, could have resulted from drastically reduced root exudates that serve as a resource for the soil microflora. According to Martikainen (2003), a shortage in root exudates can lead the rhizosphere microbes to enter a dormant, inactive stage. There were no differences between the treatments in the degradation rate of leaf litter during the 2.5-month garden experiment. In conclusion, as was hypothesized, the direct effect of wood vinegar on the soil fauna seems to be slight and short term (II, IV). Indirect effects to the soil food web due to the changes in the composition of the soil community are possible as the amount and/or quality of organic material and root exudates entering in the soil changed (Bradford et al. 2002, Marchner et al. 2004, Wardle et al. 2004). These effects probably depends on the rate and timing, as well as the type of agronomic practices of the wood vinegar application.

No differences in the numbers of nematodes were observed between control and biochar-treated pots in the greenhouse experiment (IV). Biochar had no effect on soil microbial activity at the first sampling (day 4), but a significant increase in microbial activity in the biochar-treated soils was observed 46 and 80 days after the initiation of the experiment. This enhanced microbial activity may have resulted from an increased soil organic matter content: the labile components and nutrients of the biochar may have been used by the microbes, leading to greater mineralization rates of C (IV) (Cheng et al. 2008). Furthermore, as the addition of porous biochar to soil also increases the soil surface area, soils enriched with biochar may enhance microbial growth and activity by the provision of suitable habitats for soil microbes (Lehmann 2009).

4.3 Effects of wood vinegar on plants

When sprayed on plants, wood vinegar acted as a non-selective foliar or contact herbicide by destroying virtually all growth of the aboveground parts of plants. Plants showed signs of stress and began to wither immediately after birch wood vinegar and birch tar oil applications (II). In the garden study, 40% and 60%, respectively, of the total coverage of the plants withered within the first day of application. However, the plants started to recover one month after treatment, and after 2.5 months no difference was observed in plant biomass between the variously treated plots. In the Toholampi field experiment, virtually all broad-leaved weeds withered in the wood vinegar-treated plots. At the end of the experiment, broad-leaved weeds were only present in the control pots, while in the wood vinegar-treated pots mainly couch grass (*Elymus repens*) was present. In contrast, at the end of the 3-month greenhouse experiment (IV), in which the wood vinegar was mixed in the soil and not sprayed on plant surfaces, there were no differences in plant biomass between the control and the wood vinegar-treated pots.

Given the toxicity of wood vinegar to many broad-leaved plant taxa, treatments (as a herbicide) should be applied before the emergence of cultivated seedlings. With appropriate application technology, wood vinegar has the potential to be used to control the growth of broad-leaved weeds, for example in potato and carrot fields, and in the row width of berry shrubs and fruit trees. There is evidence suggesting the suitability of wood vinegar for controlling non-indigenous species such as hogweeds (*Heracleum sp.*) (Tiilikkala et al. 2012), which are causing severe problems in Europe and North America.

Alternatively, when diluted sufficiently, wood vinegar can be applied as a soil enrichment to stimulate plant rooting and shoot growth, which was also observed in the toxicity study with *L. minor* (III). According Zulkarami et al. (2011), pyroligneous acid (wood vinegar) increased the growth and yield of rockmelon (*Cucumis melo*) plants. Similarly, Wei et al. (2009) showed that spraying with wood vinegar as foliar fertilizer increased the yield of celery (*Apium graveolens*). Wood vinegars extracted from broad-leaved trees are believed to be more efficient in increasing the growth and rooting of various plants than are wood vinegars deriving from conifers (Ogawa and Okimori 2010). An array of reports describes how wood vinegar can be used in practice, but scientific evidence gained from field experiments to support these findings is scarce (Ogawa and Okimori 2010).

4.4 Toxicity assays

Toxicity assays were performed using birch wood vinegar, because it has a greater potential in herbicidal and insecticidal use than birch tar oil. Due to its water-soluble nature, wood vinegar is relatively easy to spray in the field. The toxicity of birch wood tar to aquatic organisms was not investigated due to its viscous, sticky form, weak water solubility and because its use as a pesticide seems to be restricted only to repellent purposes. In addition, polycyclic aromatic hydrocarbons (PAHs) are usually concentrated in the tar fraction (Fagernäs et al. 2012a). Consequently, further research and product development is required before applying birch tar oil in the field. However, some PAHs (e.g. benzene), although in low quantities, were also found in the aqueous phases of wood vinegars (Fagernäs et al. 2012a). Attention must be paid to the fact that the toxicity assays in the current thesis were performed using crude wood vinegar also containing some soluble tar (II, III). Thus, pure wood vinegar is likely to be less toxic than the crude substance used in the present tests.

The toxicity of birch-derived wood vinegar was tested according to standard protocols and good laboratory practices. The studies demonstrated that aquatic organisms appear to be variably responsive to birch wood vinegar. The sensitivity of different aquatic species to birch wood vinegar was variable among the taxa, with the rank order being: *V. fisheri* (IC_{50} < 30 mg L^{-1}) < *D. magna* (EC_{50} 155 mg L^{-1}) < *L. variegates* (LC_{50} 176 mg L^{-1}) < *L. minor* (IC_{50} 229-231 mg L^{-1}) < *D. rerio*

(LC_{50} 320 mg L^{-1}) < *A. aquaticus* (LC_{50} 397 mg L^{-1}) < *S. gracilis* (LC_{50} > 381 mg L^{-1}) < *Lymnaea sp.* (LC_{50} 866 mg L^{-1}) (III). Species-specific structural, as well as functional characteristics are often associated with the bioavailability of a chemical compound (Newman and Unger 2002), which often explains the differences in the sensitivity between species. According to the *Categories of Ecotoxicity for Pesticides* (Kamrin 2000), the toxicity of a pesticide active ingredient is qualitatively classified to be very highly toxic to aquatic organisms if its LC_{50} value is less than 0.1 mg L^{-1}. Conversely, the substance is considered nontoxic if the LC_{50} value is over 100 mg L^{-1}. In the present studies, the majority of acute toxicity values for birch-derived wood vinegar exceeded this threshold. The EC_{50} value for the marine luminescent bacterium *V. fisheri* was under 30 000 µg L^{-1}, but it is unclear whether the observed effect was due to luminescence inhibition or whether the brown colour of wood vinegar resulted in the observed inhibition.

The EC_{50} value for juvenile production by the soil-inhabiting collembolan *F. candida* was 5100 mg birch wood vinegar kg^{-1} dry weight soil. No mortality (NOEC = no observed effect concentration) occurred at 3033 mg kg^{-1} dw soil. In the earthworm test, the 14-day LC_{50} for *A. caliginosa* was 6560 mg kg^{-1} (dw), the NOEC value being 2694 mg kg^{-1} (II). Most OECD countries follow the classification system according to which LC_{50} values >1000 mg kg^{-1} dw soil indicate pesticides to be practically nontoxic for earthworms (OECD 2003). As far as I am aware, literature values for the toxicity of wood vinegar to other species are not available for comparison. In general, LC_{50} values are not comparable between toxicity tests conducted in different experimental conditions and with different time scales. In the present study, the toxicity of wood vinegar for earthworms and springtails was >1000 mg kg^{-1}, indicating low toxicity of a single chemical (Russom et al. 1997).

The observed responses of wood vinegar on soil (II) and aquatic (III) organisms were attributed to the combination of chemicals present in wood vinegar. Evidently, the responses were not correlated with the concentration of the main component, acetic acid, which comprises about 12% (total weigh) of birch wood vinegar (Fagernäs et al. 2012b). It is unlikely that the effective compounds represent only a fraction of all the compounds in wood vinegar, but the effect is likely to result from the combined effects of several fractions. Furthermore, it is possible that while a particular effective compound could elicit a response in a target organism, it could be practically non-toxic for many organisms when existing in a mixture. Findings from the greenhouse experiment (IV) that (i) none of the most abundant compounds of wood vinegar were found in the waters leached through the wood vinegar-treated soils and (ii) that there were no differences in the survival of *D. magna* between waters leached through the control soil or wood vinegar-treated soils support my earlier argumentation that wood vinegar is of low environmental risk to a variety of biota.

4.5 Effects of biochar and wood vinegar on the environmental fate of glyphosate

One of the objectives of this thesis research was to explore whether biochar and wood vinegar affect the environmental fate of glyphosate in arable mineral soil (IV). The effect of plants on glyphosate leaching was also studied. Plants had a substantial effect on the leaching of glyphosate: the concentration of glyphosate in the leachates that drained through the soils in the presence of plants (*L. perenne*) was up to six times higher than in plant-free control pots, indicating that the mobility of this pesticide was enhanced by rye grass (IV). This effect was evident in soils with and without biochar or wood vinegar addition. As reported by Ruiz et al. (2008), dead roots form channels in the soil, enabling water and the soil macrofauna to reach deeper soil layers. Kjær et al. (2005) and Stone and Wilson (2006) reported that a considerable proportion of glyphosate transport can occur together with colloidal soil particles via macropores and cracks in the soil, as well as through root release and via root channels (Laitinen et al. 2007). The results of the present study lend support to these findings, suggesting that the roots of weeds may, at least to some extent, control the fate of glyphosate and its degradation products.

The duration and quantity of precipitation can also influence glyphosate leaching; when the application of glyphosate is followed by heavy rainfall, large amounts of glyphosate can be transported to deeper soil layers via soil macropores (de Jonge et al. 2000). However, in this study, sandy soils in the absence of plant (root) activities leached very low amounts of glyphosate (IV), suggesting that the risk of glyphosate leaching in soils devoid of plants or with insignificant root biomass is low, even during heavy rainfall.

As hypothesized, biochar reduced the leaching of glyphosate from the soil (IV). Due to the pooling of the glyphosate samples, the glyphosate data was not submitted to statistical analysis which undoubtedly causes uncertainty to the interpretation of results. However, the same remarkably clear trend in glyphosate leaching between the separate sampling times suggests that biochar can be effective in affecting glyphosate leaching. Furthermore, the similar response of soils with and without plants is indicative to biochar having impacts of glypohosate in the soils. When plants were present, the reduction was 18% (10 days) and 35% (44 days) after glyphosate treatment. In plant-free pots, biochar reduced the leaching of glyphosate by 40% 10 days after glyphosate treatment as compared to the control (no biochar) pots (IV). However, 44 days after glyphosate addition, glyphosate concentrations in the leachates were reduced and there were no differences between the treatments. Overall, in the absence of plants, biochar decreased the leaching of glyphosate by 27% as compared to control pots during the study.

Compared to other pesticides, glyphosate has unique sorption characteristics in soil. It has a high soil adsorption coefficient (Kd = 61 g cm^{-3}) and a very low octanol/water coefficient (Kow = 0.00033), suggesting that, despite its high water solubility (12 g L^{-1}, 25 °C), glyphosate is rather immobile and is thus unlikely to leach through the soil (Shuette 1999, Cederlund 2013). The adsorption of glyphosate is strongly dependent on the soil clay content (Dion et al. 2001) and its sorption is not, or sometimes negatively, correlated with the soil organic matter content (Gimsing et al. 2004a). However, Albers et al. (2009) reported rather high glyphosate sorption values in purified humus samples, and Shen et al. (2006) demonstrated that activated carbon has the capacity to adsorb glyphosate. Many studies have reported decreased leaching of other herbicides (Jones et al. 2011, Wang et al. 2010) after biochar addition. In line with these studies, the present research revealed that birch wood-derived biochar can influence the fate of glyphosate by reducing its likelihood of leaching from soils. This effect was evident irrespective of the presence or absence of plants. However, as biochar is produced from different parent materials and by varying pyrolysis technologies, the interactions of different kinds of biochar with soil constituents and applied agrochemical inputs are expected to be highly variable (Lehmann 2009)

Contrary to our hypothesis, the presence of biochar had no clear effect on glyphosate degradation in the soil. At the end of the study (44 days after glyphosate addition), 17–27% of glyphosate added to the pots was still present in the soils (IV). The role of biochar in the degradation of chemical pesticides is not straightforward. Several studies (e.g. Jones et al. 2011, Yang et al. 2006) have demonstrated a greater persistence and limited degradation of pesticides such as simazine and diuron in biochar-amended soils. In contrast, Zhang et al. (2005) observed that nutrients in biochar enhance the biodegradation of benzonitrile. These authors concluded that biochar can stimulate soil microbial communities by increasing the organic matter and nutrient content of soils. As the degradation of glyphosate in soils is mainly a microbiological process, microbial respiration in the soil can be used to estimate the rate of degradation of glyphosate (Von Wirén-Lehr et al. 1997). In this study, biochar also stimulated soil microbial activity during the later stages of the experiment. However, the larger and more active microbial population in the presence of biochar had no effect on glyphosate degradation, reflecting the importance of understanding the complex chemical, physical and microbiological sorption processes that evidently reduced the availability of the strongly sorbing glyphosate to microbes (Kjær et al. 2011).

The effects of wood vinegar on glyphosate leaching were inconsistent: in the presence of plants, wood vinegar increased glyphosate leaching, whereas in the plant-free pots the opposite effect was observed (IV). Soils treated with a mixture of biochar and wood vinegar showed the highest decrease in glyphosate leaching, both with and without plants. When the plants were present, the degradation of glyphosate was highest in soils treated with the biochar-wood vinegar mixture. This result was unexpected, as neither wood vinegar nor biochar, when applied alone, affected glyphosate degradation. A mechanistic understanding of these outcomes is lacking and requires further examination.

This study demonstrated for the first time that birch-derived biochar has the potential to influence the fate of glyphosate in the soil by reducing its leaching. Since the transfer of glyphosate to deeper soil layers appears to be strongly dependent on plant root release and translocation via root channels (Laitinen et al. 2007), mixing or ploughing biochar deep into the soil is likely to minimize the translocation of glyphosate from the aboveground milieu to the belowground system. This would reduce the risks of groundwater and surface water contamination by glyphosate. Obviously, due to the insignificant plant–biochar interaction, the observed treatment effects on glyphosate leaching and microbial respiration were not indirect effects via plants, but a direct outcome of the effects of biochar on these variables.

4.6 Implications for ecological risk assessment of wood vinegar

4.6.1 Use and Predicted Environmental Concentrations of wood vinegar

Effective control of perennial weeds is likely to require high doses (1300 L ha^{-1}) of wood vinegar (III). When annual crops are concerned, the required dose is about one-third (400 L ha^{-1}), and for controlling pest insects about one-tenth (130 L ha^{-1}) of the dose applied for perennial grass control (Tiilikkala and Segerstedt 2009). When wood vinegar and birch tar oil are used as repellents, the amounts ending up in the soil are insignificant, perhaps a small percentage of the BTO applied. Besides, wood vinegar doses above 400 L ha^{-1} are not realistic in practical agricultural use (Tiilikkala, K., personal communication). In weed control, for example, only target plants are treated and the lines between rows are not exposed to direct application (Tiilikkala and Segerstedt 2009). Birch wood vinegar has also been successfully used to control hogweed (*Heracleum* sp.) by destroying individual plants through spraying the leaves or injecting wood vinegar with a syringe into the hollow stem of the plant (Tiilikkala 2012).

Predicted Environmental Concentrations (PECs) are calculated by assuming a soil bulk density of 1.5 g cm^{-3} and a mixing depth of 5 cm for applications to the soil surface (FOCUS 2006). The mass of wood vinegar is 980 mg ml^{-1} (400 L ha^{-1} = 392 kg ha^{-1}) (Pasanen 2006). When assuming a realistic PEC_{soil}, the total plant coverage must be taken into account. If wood vinegar is applied in early spring, up to 90% of the used volume may enter the soil surface. Based on these data, the PEC_{soil} for wood vinegar after a single application (400 L ha^{-1}) was calculated in this study as follows:

PEC_{soil} = 392 000 g ha^{-1} * (1-0.1) / (100 * 5 cm * 1.5 g cm^{-3}) = 470.4 mg kg^{-1}

However, when used for broad-leaved weed control, only 10% is assumed to end up in the soil, resulting in a PEC of 53.1 mg kg^{-1}. When wood vinegar is applied as an insecticide, a 10% dilution is usually used (Tiilikkala and Segerstedt 2009) and the volume entering into soil is only 1–30% of the total volume, producing a wood vinegar concentration of 4.7–14.1 mg kg^{-1} in the soil.

4.6.2 Exposure assessment of wood vinegar as a mixture

Risk characterization based on toxicity to exposure ratio

The initial risk characterization was performed by means of toxicity-to-exposure ratios (TER). TER is used as an indicator of risk in the assessment process (EC 2003). TER value for wood vinegar on earthworms (II: LC_{50} 6560 mg kg^{-1}) was calculated according the following formula (SANCO 2002):

TER_{acute} = 6560 mg kg^{-1} / 470.7 mg kg^{-1} = 13.9 → acceptable risk

In the *Council Directive* concerning the marketing of plant protection products (91/414/EEC, Annex VI), boundary values are presented for the TER to account for uncertainties (e.g. lab to field or tested species vs. all species). Annex VI (91/414/EEC) specifies the decision rule: TER ≥ 10 for acute risks and ≥ 5 for long-term risks. TER_{acute} >10 for earthworms (91/414/EEC) indicates that the use of birch wood vinegar is acceptable with no obvious risk to soil organisms. Boundary values act as a safety margin: if the values are under the boundary limit, a closer risk characterization is required (Mattsoff 2005). To be on the safe side, a reproduction assay (30 d) was carried out using the collembolan *F. candida* and resulted in an EC_{50} value of 5100 mg kg^{-1} for juvenile production (II). The critical TER value for arthropods according to 91/414/EEC is 5. To predict the risk caused by wood vinegar to this non-target arthropod, the TER value was calculated as follows:

$TER_{chronic}$ = 5100 mg kg^{-1} / 470.7 mg kg^{-1} = 10.8 → acceptable risk

According to these TER values, wood vinegar does not cause a risk to soil organisms when the applied doses are below 400 L ha^{-1}. The results of the present laboratory and field studies, in which birch wood vinegar had no effects on enchytraeids, nematodes or soil microbes, even when applied in large quantities (500–2000 L ha^{-1}) (II), support the conclusion that wood vinegar poses a low environmental risk.

PNEC values and ecological risk caused by wood vinegar

PNEC –values for wood vinegar were calculated for aquatic and soil environment. The $PNEC_{aqua(freshwater)}$ was calculated according the NOEC value (82 mg L-1) for *L. variegatus* (III), which was observed to be the most sensitive aquatic organism to birch wood vinegar (Fig. 2). When calculating the $\mathrm{PNEC}_{\mathrm{aqua(freshwater)}}$ for wood vinegar, an assessment factor of 100 was applied, as acute LC_{50} or NOEC values are available for several aquatic organisms (III) (Fig. 2), but only one IC_{50} value from a long-term test:

$\mathrm{PNEC}_{\mathrm{aqua\ (freshwater)}} = 82\ \mathrm{mg\ L}^{-1} / 100 = 0.82\ \mathrm{mg\ L}^{-1}$

An assessment factor 10 was used for calculating the PNEC for soil organisms, as a NOEC value is available for two soil organisms, and several field and laboratory examinations have demonstrated no effect on soil microbes, nematodes or enchytraeids, even at high wood vinegar application rates (500–2000 kg ha^{-1}) (II). The NOEC (2694 mg kg^{-1}) for *A. caliginosa* was used as a source value (II).

$\mathrm{PNEC}_{\mathrm{soil}} = 2694\ \mathrm{mg\ kg}^{-1} / 10 = 269\ \mathrm{mg\ kg}^{-1}$

The ecological risk of wood vinegar in the terrestrial environment was also estimated numerically using the hazard quotient (HQ) approach. NOEC values for *F. candida* (3033 mg kg^{-1}) and *A. caliginosa* (2694 mg kg^{-1}) were used (II) to derive HQ value for wood vinegar according the following formula (SANCO 2002):

$\mathrm{HQ} = 470.7\ \mathrm{mg\ kg}^{-1} / (3033\ \mathrm{mg\ kg}^{-1} / 10) = 1.55$ → acceptable risk

$\mathrm{HQ} = 470.7\ \mathrm{mg\ kg}^{-1} / (2694\ \mathrm{mg\ kg}^{-1} / 10) = 1.75$ → acceptable risk

Figure 2. NOEC values (mg L^{-1}) of wood vinegar for the tested terrestrial and aquatic species (M. Hagner et al. unpublished). Based on the results of publications II and III.

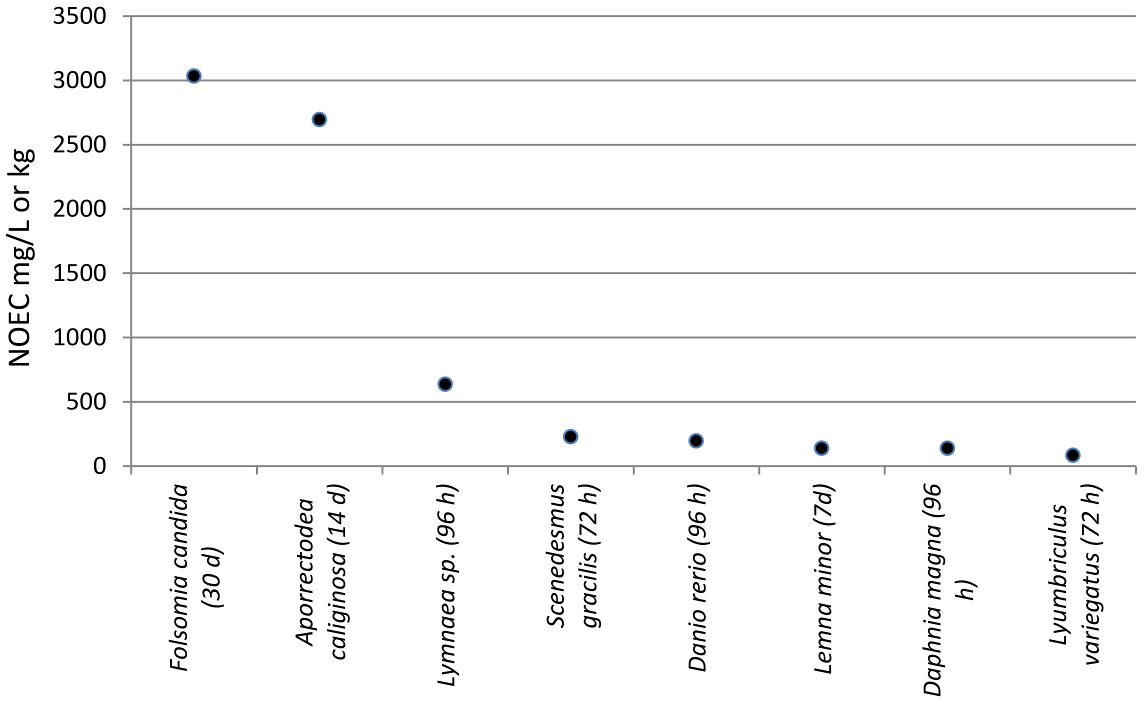

Table 2. $PNEC_{aqua}$ and $PNEC_{soil}$ values (from literature) versus volume and PEC values (weight total %) for the five most common substances of birch wood vinegar in soil immediately after application.

Compound	% of wood vinegar	$PNEC_{aqua}$ mg L^{-1}	$PNEC_{soil}$ mg kg^{-1} dw	Concentration after wood vinegar application mg kg^{-1}	PEC / $PNEC_{soil}$
Acetic acid	9.0-12.0	3.058[1]	0.47[1]	56.5	120.2
Methanol	1.5-1.8	2.375[2]	0.348[2]	8.5	24.4
1-Hydroxy-2-propanone	0.7-1.1	-	-	5.17	-
Acetone	0.11-0.5	10.6[3]	29.5[1]	2.35	0.08
Furfural	0.2-0.36	0.033[4]	0.014[4]	1.69	120.7

[1] ECHA 2013
[2] Uuksulainen et al. 2008
[3] Staples 2000
[4] EU 2008

Usually HQ values less than 1.0 are considered to indicate an acceptable risk, whereas HQ $\geq$ 1.0 indicates an unacceptable risk. If the HQ ratio of 2 for arthropods is exceeded, a litter test is required (Mattsoff 2005). The Guidance Document on Terrestrial Ecotoxicology Under Council Directive 91/414/EEC (SANCO 2002) concludes that if the HQ (where the correction factor of 10 has been applied) is less than 2, no further assessment is required.

As a result, the HQ value (<2) indicates that there is no ecological threat to the soil arthropods when using birch wood vinegar at an application rate of less than 400 L ha^{-1}. To obtain a HQ value of wood vinegar <1, its application dose must be restricted to 230 kg ha^{-1} to achieve a PEC below 269 mg kg^{-1}. In this study, toxicity values (LC_{50}, EC_{50}, NOEC) were measured using crude wood vinegar, in which the organic matter content is twice as high as in pure wood vinegar. Consequently, when using pure wood vinegar, the toxicity values will be higher, resulting HQ values <1, even at an application rate of 400 L ha^{-1}. In our studies, the effects of wood vinegar on the environment were assessed under realistic semi-field and field conditions, and no significant effects on soil properties, the studied soil organisms, soil functions or plant productivity were noted.

4.6.3 Compound-specific exposure assessment of wood vinegar in soil

This chapter discusses the most abundant components of wood vinegar that may produce the observed toxic effects on organisms tested in this thesis study. Compound-specific assessment is based on the idea that all components in the mixture behave as if they are simple dilutions of one another, having an identical mechanism of action (EC 2010). Pyrolysis conditions and parent materials can cause batch-to-batch variation in the composition of wood vinegar (Lehmann 2009). When using birch (*Betula pendula*) as a parent material, wood vinegar produced with slow pyrolysis contains about 70–75% water and the amount of organic matter is then 25–30% (Fagernäs et al. 2012b). In their studies, Fagernäs et al. (2012b) compared the variability of birch wood vinegar from different retorts and noted only slight variation between the cohorts or the separate batches of a single producer.

The five most abundant components of birch-derived wood vinegar are: acetic acid, methanol, 1-hydroxy-2-propanone, acetone and furfural (Fagernäs et al. 2012b). In this chapter, an initial assessment of the environmental risk of these five abundant compounds of wood vinegar was performed by comparing their predicted environmental concentration in the soil after wood vinegar addition to the $PNEC_{soil}$ values found from the literature to calculate PEC/PNEC ratios (Table 2). If the PEC exceeds the PNEC, i.e. the ratio is more than one, there is considered to be a risk of environmental damage, and further risk characterization is needed. A ratio of less than one indicates a low environmental risk (EC 2003).

The $PEC/PNEC_{soil}$ ratio of 1-hydroxy-2-propanone (e.g. hydroxyacetone, hydroxypropanone) could not be calculated, as no $PNEC_{soil}$ values were found in the literature (Table 2). 1-Hydroxy-2-propanone comprises 0.7–1.1% of wood vinegar (Fagernäs et al. 2012b). Immediately after the application of wood vinegar, its concentration in soil is at maximum 5.17 mg kg^{-1} (470.7 mg kg^{-1} * 0.011). 1-Hydroxy-2-propanone has been reported to be a safe flavouring agent on the flavouring substances list of the Flavor and Extract Manufacturers Association, with a maximum usage level in soft candies, for example, of 50 ppm (50 mg kg^{-1}) (Smith et al. 2009). The LD_{50} (oral) of 1-hydroxy-2-propanone for rats is 2200 mg kg^{-1} and the LC_{50} for fish (*Leuciscus idus*) (96 h) varies from 4600 to 10 000 mg L^{-1} (Smith et al. 2009). It is readily biodegradable (95%, 20 d), and accumulation in organisms is not to be expected (log P_{ow} -0.78) (EPA 2013). Based on these data, 1-hydroxy-2-propanone entering the soil in wood vinegar application is unlikely to cause an environment risk.

The concentration of acetone in wood vinegar varies from 0.11 to 0.5% (Fagernäs et al. 2012b). The maximum acetone dose in the soil after wood vinegar application is 2.35 mg kg^{-1} (470.7 mg kg^{-1} * 0.005). Acetone is soluble in water (logP_{ow} -0.24) and does not bind to soil particles or accumulate in living organisms. In soil and water, acetone is rapidly (1 to 14 days) degraded by microbes. Based on the results from toxicity tests with a wide variety of aquatic and terrestrial species, acetone is believed to be only slightly toxic (OECD 1999). The LC_{50} for aquatic invertebrates ranges from 2100 mg L^{-1} to 16 700 mg L^{-1}. The chronic NOEC for Daphnia is 1660 mg L^{-1} (OECD 1999). $PNEC_{aqua\ (freshwater)}$ for acetone is 10.6 mg L^{-1} (Staples 2000). Several $PNEC_{soil}$ values for acetone were found from literature, of which 29.5 mg kg^{-1} was the most commonly used (ECHA 2013). Here, the $PNEC_{soil}$ was compared to the PEC of acetone in soil after wood vinegar application to calculate the PEC/PNEC ratio, which yielded a ratio of 0.08. This margin of exposure is less than one; acetone was therefore considered to have a low environmental risk potential.

As $PEC/PNEC_{soil}$ ratios of acetic acid, methanol and furfural in the soil after wood vinegar application were found to exceed the limit value of >1 (Table 2), there are considered to be unacceptable effects on organisms. Thus, the environmental risks of these compounds are separately assessed in the following sections of this thesis.

Acetic acid

Acids are the most common substances (35–40%) in the organic part of birch wood vinegar, of which about 85% is acetic acid (Fagernäs et al. 2012b). The log P_{ow} value of -0.17 for acetic acid indicates that it is water soluble and not bioaccumulative. Acute toxicity values (LC_{50}) of acetic acid for fish are reported to range between 45 mg L^{-1} (*Oncorhynchus mykiss*) and 410 mg L^{-1} (*Cyprinus orfus*) (ECHA 2013), indicating acetic acid to be only slightly toxic or non-toxic to fish. The $PNEC_{aqua(freshwater)}$ of acetic acid is 3.058 mg L^{-1} and the $PNEC_{soil}$ is 0.47 mg kg^{-1} dw (ECHA 2013).

Several plant protection products with acetic acid as an active substance are commonly applied in EU, such as Cooper (Berner Ltd.). In this product, the acetic acid concentration in the applied dilution is 62 g L^{-1}, and the recommended dose in the field is 1–1.25 dL m^{-2}. When used in weed control Cooper and wood vinegar are usually applied in the field by spraying. When spraying Cooper in early spring, in the worst case 90% of the product can enter the soil (equivalent to 74–90 mg kg^{-1} acetic acid). Birch wood vinegar contains about 88–107 g L^{-1} acetic acid. The recommended concentration of wood vinegar in field use is less than 400 L ha^{-1}, i.e. less than 56.5 mg kg^{-1} (470.7 mg kg^{-1} * 0.12) acetic acid in soil immediately after spraying. Thus, the acetic acid concentration in soil treated with wood vinegar is lower than when using Cooper. The biological oxygen demand (BOD5) for acetic acid is 0.88 g g^{-1} and BOD/ThOD (theoretical oxygen demand) is 36–80% (5 d), indicating rapid degradation in the environment. A large number of studies have shown that acetic acid biodegrades readily under both aerobic and anaerobic conditions in terrestrial and aquatic environments (e.g. Howard et al. 1992, Kameya et al. 1995). Based on these data, acetic acid in wood vinegar is not assumed to cause environmental risks.

Methanol

Methanol is the second most abundant substance, forming 1.5–1.8% of the organic fraction of wood vinegar (Fagernäs et al. 2012b). The half-life of methanol depends on numerous factors, including the nature and quantity of release, and the physical, chemical and microbiological characteristics of the impacted matrix. The log P_{ow} of methanol is 0.8–2.75, inferring its bioaccumulation and adsorption on soil particles to be minimal due to its low lipophilicity (Mackay et al. 2006). The BOD5 value is 0.6–1.1 g g^{-1}, which is 40–73% of the theoretical oxygen demand. Methanol biodegrades rapidly, its half-life being 1 to 7 days in the soil and in surface and groundwater, and about 18 days in the atmosphere. Methanol is unlikely to accumulate in the soil, air, surface water or groundwater (Malcom Pirnie 1999).

The acute toxicity (LC_{50}) values of methanol for aquatic species vary from 100 mg L^{-1} to 29 000 mg L^{-1} (Ewell et al. 1986, EPA 2013). Most of the LC_{50} values are above 1000 mg L^{-1} (“relatively harmless”) and only a few are between 100 and 1000 mg L^{-1} (practically non-toxic), which indicates that methanol is essentially non-toxic to aquatic organisms. However, a low chronic NOEC of methanol (90 d, 23.75 mg L^{-1}) for fish was reported by Kaviraj et al. (2004). Preliminary PNEC values for methanol in the aquatic and soil environment have been reported by Uuksulainen et al. (2008). They calculated the $PNEC_{aqua}$ using the chronic NOEC value of 23.75 mg L^{-1} for fish by dividing the NOEC by an assessment factor of 10, resulting in a $PNEC_{aqua}$ of 2.375 mg L^{-1}. In addition, Uuksulainen et al. (2008) calculated a $PNEC_{soil}$ value of 0.348 mg L^{-1} from the chronic $PNEC_{aqua}$ value using the equilibrium partitioning method due to the lack of research results concerning the effects of methanol on soil organisms. However, Stantec Consulting Ltd. (2006) analysed the toxicity of methanol to two soil invertebrate species, the earthworm *Eisenia andrei* and the springtail *F. canadida*. EC_{25} values for reproduction for these two invertebrates ranged from 2842 mg kg^{-1} to 13 323 mg kg^{-1}. These values were used in the present study to calculate a new $PNEC_{soil}$ value for methanol. Using the assessment factor of 100, the resulting $PNEC_{soil}$ was 28.4 mg kg^{-1} (2842 mg kg^{-1} / 100).

Wood vinegar contains 1.8% methanol (at most), i.e. less than 8.5 mg kg^{-1} (470.7 mg kg^{-1} * 0.018) of methanol enters the soil immediately after application. This is less than the calculated $\mathrm{PNEC_{soil}}$ value of 28.4 mg $\mathrm{kg^{-1}}$ for methanol, resulting in a $\mathrm{PEC/PNEC_{soil}}$ ratio of less than one. In addition, this new $\mathrm{PNEC_{soil}}$ value has been calculated using chronic NOEC values. As methanol biodegrades rapidly in soil (Malcom Pirnie 1999), its concentration after wood vinegar application is not assumed to cause chronic effects. As a consequence, methanol entering in the soil due the wood vinegar application is therefore considered to have a low environmental risk potential.

Furfural

Furfural is a relatively volatile compound (Henry's Law constant 3.8 x 10^{-6} atm-cu m/mol) and only slightly soluble in water (83 g $\mathrm{L^{-1}}$). It is rapidly degraded in the atmosphere (<1 day) by reactions with hydroxyl radicals (EC 2008). On the basis of the low log P_{ow} value of 0.41, furfural is not expected to bioaccumulate; it is highly mobile in soil and prone to leaching into groundwater. In the aquatic environment, nearly 100% degradation occurs in 30 days. Furfural is also readily biodegradable in soil (EC 2008).

Furfural is widely used. In the EU it is mostly applied in the production of furan derivatives and, for example, in weed killers, fungicides and extraction solvents (EC 2008). The EU Risk Assessment Report describes the effect of furfural on organisms representing various trophic levels (EC 2008). Acute toxicity endpoint values for aquatic invertebrates are in the range of 10.5 to 32 mg $\mathrm{L^{-1}}$, indicating furfural to be moderately toxic to aquatic species following short-term exposure. Longer exposure may cause toxic effects at relatively low concentrations. The lowest long-term NOEC was found for the zebra fish, *Brachydanio rerio*: the NOEC for the behaviour and morphology of fish larvae was 0.33 mg $\mathrm{L^{-1}}$. Applying an assessment factor of 10, this corresponds to a PNEC value of 33 µg $\mathrm{L^{-1}}$ for aquatic organisms. No toxicity data are available for the toxicity of furfural to soil organisms. This is considered as a serious limitation for a compound with a relatively high vapour pressure (SCHER 2008). The equilibrium partitioning method leads to a $\mathrm{PNEC_{soil}}$ value of 0.014 mg $\mathrm{kg^{-1}}$ wet weight when using chronic values from aquatic toxicity tests as source data (EC 2008).

The furfural concentration in birch-derived wood vinegar ranges between 0.2 to 0.36% (Fagernäs et al. 2012b). The total amount of furfural entering soil at a wood vinegar application rate of 400 L $\mathrm{ha^{-1}}$ corresponds to 1.69 mg $\mathrm{kg^{-1}}$ (~0.00146 ml $\mathrm{kg^{-1}}$). This amount is over a hundred times greater than the $\mathrm{PNEC_{soil}}$ (0.014 mg $\mathrm{kg^{-1}}$) in the EU. Africa's leading sugar producer, Illovo Sugar, sells Crop Guard® for the control of nematodes on crops**.** Crop Guard® contains furfural (900 g $\mathrm{kg^{-1}}$) and the recommended application rate varies from 50 to 75 L $\mathrm{ha^{-1}}$. This results in a furfural dose of up to 0.1 ml kg $^{-1}$ (~116 mg $\mathrm{kg^{-1}}$) immediately after application, which is a hundred times greater than the amount entering the soil when wood vinegar is applied. Similar furfural doses are also applied in the United States for the control of nematodes in turf grass, peanut and vegetable crops, among others (El-Mougy et al. 2008).

The Ministers of the Environment and of Health in Canada (2011) have recently published a screening assessment for furfural in which $\mathrm{LC_{50}}$ values for soil organisms are reported: an $\mathrm{LC_{50}}$ of 406.18 mg $\mathrm{kg^{-1}}$ (14 d) for the earthworm *E. foetida* and an NOEC value of 37.5 mg $\mathrm{kg^{-1}}$ (28 d) for the arthropod *F. candida*. In this study, a new $\mathrm{PNEC_{soil}}$ value was calculated for furfural by using the NOEC value from chronic toxicity tests with *F. candida*. Applying an assessment factor of 10 produces a $\mathrm{PNEC_{soil}}$ of 3.75 mg kg $^{-1}$ (37.5 mg $\mathrm{kg^{-1}}$ / 10). This value is not exceeded when using wood vinegar, the resulting $\mathrm{PEC/PNEC_{soil}}$ ratio being less than one, which indicates that furfural entering in the soil due to wood vinegar application has a low environmental risk potential.

In the present studies (II, III), wood vinegar treatments resulted in a furfural concentration of up to 7.2 mg kg^{-1} in soil with no effect on nematodes, enchytraeids or soil microbes. Furthermore, in study IV, no furfural remains were observed in leachate waters collected 5 days after wood vinegar addition, despite the considerable application rate (2000 L ha^{-1}). This indicates a low leaching risk of furfural after wood vinegar addition.

Fagernäs et al. (2012b) investigated the effect of aging on the composition of wood vinegar: 6 months of storage reduced the amount of furfural by up to 30%. Despite this reduction, the repellent efficiency of wood vinegar against snails was unaffected (M. Hagner et al., unpublished). If necessary, the aging process will reduce the furfural content of wood vinegar.

To summarize, applying wood vinegar in the field at 400 L ha^{-1}, the initial maximum concentration in the soil after spraying is 470.7 mg kg $^{-1}$. The wood vinegar concentration in soil will rapidly decrease as a result of microbial degradation, volatilization and leaching. The half-lives of the main components of wood vinegar (acetic acid, methanol, acetone, 1-hydroxy-2-propanone and furfural) in soil are less than one month. Most components of wood vinegar are also rapidly degraded in aqueous solutions and in the atmosphere. Concentrations of the most abundant wood vinegar compounds (acetic acid, methanol and furfural) may exceed the current $\mathrm{PNEC_{soil}}$ values found in the literature. These previous $\mathrm{PNEC_{soil}}$ values were calculated from aquatic PNEC values using a partitioning coefficient. The equilibrium partitioning method (EqP) is commonly used to estimate terrestrial PNEC values from aquatic PNEC values, when insufficient soil toxicity data are available (EU 2003). Van Beelen et al. (2003) emphasized that when the EqP method is performed to estimate the terrestrial values from aquatic toxicity data, the terrestrial values can be over- or underestimated. Thus, new $\mathrm{PNEC_{soil}}$ values were calculated for furfural and methanol using realistic values from recently conducted toxicity studies with soil organisms, and these showed that the EqP method has resulted in a significant overestimation of the $\mathrm{PNEC_{soil}}$ values of methanol and furfural. When comparing the estimated PEC values of furfural and methanol in soil after wood vinegar application with the newly derived $\mathrm{PNEC_{soil}}$ values, it can be concluded that these compounds do not cause a risk to the environment when ending up to soil after wood vinegar application (<400 L ha^{-1}). The acetic acid concentration entering the soil is less than when using acetic acid-containing substances approved for herbicidal use in the EU. It is to be acknowledged that there may be interactions between the various compounds within a mixture. For example, toxicokinetic interactions between compounds may affect the observed overall toxicity of a mixture (IGHRC 2009).

4.6.4 Risk of wood vinegar to the aquatic environment

The persistence of a pesticide in the soil is of great importance in pest management and environmental pollution. The metabolic fate of pesticides is dependent on pesticide characteristics (e.g. hydrophilicity, *K*ow), abiotic environmental conditions (e.g. temperature, moisture, pH), the microbial community and plant species, and biological and chemical reactions (Van Eerd et al. 2003).

On the basis of the lowest NOEC value (*L. variegates;* 82 mg L^{-1}) from aquatic toxicity tests and applying an assessment factor of 100, the $\mathrm{PNEC_{aqua(freshwater)}}$ value of 0.82 mg L^{-1} for wood vinegar was derived. It is to be noted that, as slow pyrolysis originated wood vinegar is readily biodegradable (Blin et al. 2007), the long-term exposure of aquatic organisms to wood vinegar is not realistic, and the assessment factor may be lowered.

It is challenging to assess the runoff and leaching of wood vinegar to aquatic systems because it consists of hundreds of chemicals with different physical and chemical properties. There is no single active ingredient that can be used as an indicator of the leaching risk. Wood vinegar is not intended to be sprayed directly onto aquatic systems, and buffer zones must be maintained between fields and watercourses until transport to waters has been properly examined. The concentrations of wood vinegar entering aquatic systems are thus minimal and buffer zones established for pesticides containing acetic acid are also sufficient for wood vinegar. The present findings (IV) that none of the 14 quantitatively most abundant compounds in wood vinegar were detected in water leachate, despite the substantial application rate of wood vinegar (2000 L ha^{-1} + 500 L ha^{-1}) to soils, and that there were no differences in the survival of the water flea *D. magna* between control waters or waters leached through differentially treated soils, support my earlier claims that wood vinegar is unlikely to cause a risk in the aquatic environment.

5. CONCLUSIONS AND FUTURE CHALLENGES

There is little doubt that pesticide application should be reduced, and replacement substances such as low-risk pesticides as well as biological control methods and technologies should be considered in the first place. Scientific results concerning the efficacy of botanical products in sustainable plant protection and integrated pest management are needed.

The use of wood vinegar, a liquid produced through the distillation or pyrolysation of organic materials, has rapidly increased in Asian countries, where wood vinegar is believed to act as a biocide against microorganisms, weeds and insects. Despite this, scientific evidence for the efficacy of wood vinegars in pest control is scarce (Tiilikkala et al. 2010). The present studies provide strong evidence for the potential of birch-derived pyrolysis liquids to be applied as an effective, non-costly and environmental friendly method against molluscs (*A. arbustorum, A. lusitanicus*) (I).

Previous studies on wood vinegar application have reported very little about its toxic effects in the environment. In the studies reported in this thesis, soil organisms were observed to be more tolerant of wood vinegar than aquatic organisms (II, III). Results from ecotoxicological studies (II, III, IV) were used to derive toxicity exposure ratio (TER) and hazard quotient (HQ) values according to the guidance documents on EU regulations to indicate the environmental risk caused by wood vinegar. Both values indicated that there is no ecological threat to the soil when using birch wood vinegar at an application rate of less than 400 L ha^{-1}. As a consequence, there is no need for the separate ecotoxicological testing of each compound in wood vinegar. This would indeed make risk assessment unmanageable and might result in a different final conclusion than when assessing it as a mixture, in which case any interactions between the compounds are captured in the observed responses of the exposed organisms.

The findings that none of the most abundant compounds of wood vinegar were detectable in leachates, and that there were no differences in the survival of *D. magna* between control waters or waters leached through soils treated with wood vinegar (IV), support my earlier claims (I, II, III) that wood vinegar is of low environmental risk and is rapidly degraded through microbial activity. As wood vinegar is only slightly toxic or non-toxic to most aquatic and soil organisms (II, III), the environmental risk caused by conventional synthetic pesticides could be reduced by including wood vinegar as part of a pest control methodology. Wood vinegar could act as a complementary pesticide in an IPM strategy, as in all cases its efficiency is insufficient to meet pest control requirements.

In herbicidal use, wood vinegar acts as a foliar or contact herbicide and has provided satisfactory results, particularly in the control of broad-leaved weeds such as *Chenopodium album*, *Stellaria media* and *Heracleum persicum* (Tiilikkala and Segerstedt 2009, Tiilikkala 2012). Moreover, it has been suggested that wood vinegar could be mixed with synthetic herbicides (Rico et al. 2007) or insecticides (Kim et al. 2008) to improve their effects. Thus, it could be possible to reduce the volume of synthetic pesticides applied by improving their efficiency through using wood vinegar as an additive. However, this suggestion requires thorough scientific investigation before implementing in the field.

Despite the large number of toxicity studies conducted on wood vinegar, the requirements for REACH registration are still in their infancy. It is likely that the use of wood vinegar under field conditions will result in exposure to honeybees. As a consequence, both acute oral and contact toxicity tests on honeybees must be conducted according to OECD guidelines. Because *A. caliginosa* was observed to be the most sensitive soil organism, it might also be necessary to test the effects on the reproduction of earthworms according OECD guidelines. Moreover, the effects of wood vinegar on human health must be assessed according to valid, widely used methods.

Biochar, another slow pyrolysis product, can also play a role in pesticide risk reduction, particularly in preventing the contamination of the aquatic environment. Many studies (e.g. Jones et al. 2011, Wang et al. 2010) have reported reduced leaching of herbicides after the addition of biochar to or on the soil. In line with this, the present study suggests for the first time that birch wood-derived biochar may influence the fate of glyphosate by reducing its likelihood of leaching out of the soil ecosystem (IV). The transfer of glyphosate to deeper soil layers seems to be strongly dependent on its release through plant roots and/or translocation via root channels. Mixing or ploughing biochar deep into the soil and the establishment of exclusion areas around fields is likely to minimize the translocation of glyphosate to groundwater and surface waters.

It is not yet clear how various pesticides react to biochar addition. It is possible that biochar could change the way a given pesticide behaves in the soil. In the case of pesticides acting at the soil level, their activity could be reduced by biochar addition, and more pesticides may be needed to produce the same level of pest control, which is not in accordance with sustainable agriculture. Interestingly, soils treated with a mixture of biochar and wood vinegar were found to leach less glyphosate than soils treated with biochar or wood vinegar alone, or control soils without additions. This was evident whether plants were present or not. Due to the pooling of the glyphosate samples, the glyphosate data was not submitted to statistical analysis which undoubtedly causes uncertainty to the interpretation of results. However, the same remarkably clear trend in glyphosate leaching between the separate sampling times suggests that biochar can be effective in affecting glyphosate leaching. Furthermore, the similar response of soils with and without plants is indicative to biochar having impacts of glypohosate in the soils. The reason why the biochar-wood vinegar mixture retained glyphosate better than soils in which the pyrolysis products occurred separately remains to be determined.

The sorption of pesticides to biochars can lead accumulation of pesticides in surface soils (Jones et al. 2011). Pesticides bound to biochar are not likely to be bioavailable and may actually minimize root uptake and further contamination of the food chain (Wang et al. 2012, Yang et al. 2010, Yu et al. 2009). However, enhanced biodegradation of a pesticide has also been observed in the presence of biochar (Zang et al. 2005). Biochar may stimulate biodegradation by supplying nutrients for microbial activity and growth. However, in this study the larger and more active microbial population in soils with biochars had no effect on glyphosate degradation, reflecting the importance of understanding the complex chemical and/or soil-specific sorption processes that evidently reduce the availability of the strongly sorbing glyphosate to microbes (Kjær et al. 2011).

Based on the results of this thesis, birch derived slow pyrolysis liquids and biochar appear to have potential to be used in sustainable plant protection and integrated pest management as they have several application possibilities in large-scale agriculture and also in private gardens. However, as pyrolysis liquids and biochar are produced from various parent materials and by varying pyrolysis technologies (Oasmaa et al. 2010), their interactions with soil constituents and applied agrochemical inputs are expected to be highly variable.

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