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255

Marina Himanen

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UNIVERSITY OF JYVÄSKYLÄ

JYVÄSKYLÄ 2013

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ABSTRACT

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Diss.

Composting is an aerobic technology for processing organic waste into a valuable product that improves soil quality and supports plant development. However, immature compost may impair plant growth, causing e.g. economic losses. Low-weight carboxylic acids (LWCA), with an aliphatic chain less than 6 carbons play a key role in phytotoxicity of immature composts. The role of LWCA in phytotoxicity of compost was studied in composting experiments and short-term and subchronic plant assays using cress *Lepidium sativum*. Results of the experiments showed that a significant amount of LWCA entered the process with the feedstock. In the composts, where aerobic and anaerobic municipal wastewater sludges were used as a feedstock, the concentrations of LWCA decreased significantly during the first two weeks of composting. On the contrary, in biowaste composts LWCA accumulated to concentrations from 2 to 3 times higher than the initial concentrations. In experiments, where the effect of aeration was studied, accumulation of LWCA was higher in compost aerated at high rate (5 l min^{-1}) than at low rate (0.5 l min^{-1}). Application of a calcium-based additive caused a temporary boost in the concentration of acetic acid during the first days decreasing significantly over the first week. In all experiments concentrations of LWCA decreased by 60 % to 99 % from the initial values stabilizing at 100–800 mg kg^{-1} dry matter (water-extractable) and 800–1500 mg kg^{-1} dry matter (alkaline-extractable), thus forming a pool in mature composts. Depending on the process design the phytotoxic period of compost lasted from 2 to 24 weeks. Stronger aeration and application of the calcium-based additive significantly shortened it. Dose-response experiments with pure LWCA and their mixtures allowed defining a safe level (EC10), which was about 800 mg kg^{-1} dry matter. Concentrations of LWCA in immature composts were well above the EC10 value, while the concentrations in mature compost were predominantly below it. Correlation analysis of LWCA concentrations and phytotoxicity of composts showed a moderate dependence between these variables, suggesting the possibility of some other causes of compost phytotoxicity than LWCA.

Keywords: Biowaste; compost; compost additives; low-weight carboxylic acids; municipal sludge; phytotoxicity.

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CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The thesis is based on five original papers, which will be referred to in the text by their Roman numerals I-V. I made a significant contribution in planning of the experiments, performed majority of the experimental work described in papers I-III, supervised experimental work and conducted data analysis in IV and V. I also wrote the first drafts of all papers, which were completed in co-operation with the co-authors.

I Himanen M. & Hänninen K. 2011. Composting of bio-waste, aerobic and anaerobic sludges – Effect of feedstock on the process and quality of compost. *Bioresource Technology* 102: 2842–2852.

II Himanen M. & Hänninen K. 2009. Effect of commercial mineral-based additives on composting and compost quality. *Waste Management* 29: 2265–2273.

III Himanen M., Latva-Kala K., Itävaara M. & Hänninen K. 2006. A method for measuring low-weight carboxylic acids from biosolid compost. *Journal of Environmental Quality* 35: 516–521.

IV Himanen M., Prochazka P., Hänninen K. & Oikari A. 2012. Phytotoxicity of low-weight carboxylic acids. *Chemosphere* 88: 426–431.

V Himanen M., Hänninen K. & Oikari A. 2012. Low-weight carboxylic acids as potential risk in phytotoxicity of processed biomasses. Submitted manuscript.

ABBREVIATIONS

AnS	anaerobic sludge from municipal wastewater treatment
AR	aeration rate
AS	aerobic sludge from municipal wastewater treatment
BW	at-source separated biowaste
dm	dry matter
EC	effective concentration
ECN	European Composting Network
GC-FID	gas chromatography – flame ionization detector
HPLC-APCI-MS	high-performance liquid chromatography-atmospheric pressure chemical ionization mass spectrometry
LWCA	low-weight carboxylic acids
OM	organic matter
PBGM	peat-based growth media
IC-CD	ion chromatography with conductivity detector

1 INTRODUCTION

1.1 Composting – technology for recycling biowaste and sludges

1.1.1 Biowaste and wastewater sludge recycling in Finland

Nowadays composting is an important technology for the recycling of biodegradable organic matter. It is widely used for utilization of at-source separated biowaste and sewage sludges from municipal wastewater treatment.

Driven by changes in legislation, at-source separation of biowaste has become an obligatory part of a modern waste treatment system in EU countries. In 2008 the annual generation of biowaste in the EU ranged from 118 to 138 Mt, out of which around 88 Mt originated from municipal waste and between 30 to 50 Mt from industrial sources such as food processing (Anon. 2010a). In Finland the amount of collected biowaste from municipal waste was constantly growing from 165 000 tons in 2002 to 300 400 tons in 2010 (Fig. 1). The annual collection rates of municipal wastewater sludge slightly decreased from 161 500 in 2002 to 142 700 tons in 2010 (Statistics Finland 2012).

Composting has been established as a common practice for the recycling of biowaste and sewage sludge. According to the data provided by the European Composting Network (ECN) (2008) production of compost in EU countries in 2005 was more than 13 million tons, including compost from the biodegradable fraction of MSW and sewage sludge. In Finland data on compost production rates are lacking, however, it is documented that recycling rates for biowaste grew from 85 % in 2002 to 98 % in 2010 and recycling rates for municipal sewage sludge were 95 %–99 % in 2002–2010 (Fig. 1).

Compost as a product is used in two main ways: as such in a form of soil improver or organic fertilizer, or as a component of the growing media. In Finland amount of soil improvers produced from compost grew from 430 000 m^3 in 2005 to 555 000 m^3 in 2010 (Fig. 1) (Anon. 2012a). The amount of soil improvers produced from stabilized activated sludge or sewage sludges

utilized as such increased significantly from 20 000 m^3 in 2005 to 114 000 m^3 in 2010. The products are used mainly in landscaping and in agriculture (Fig. 2).

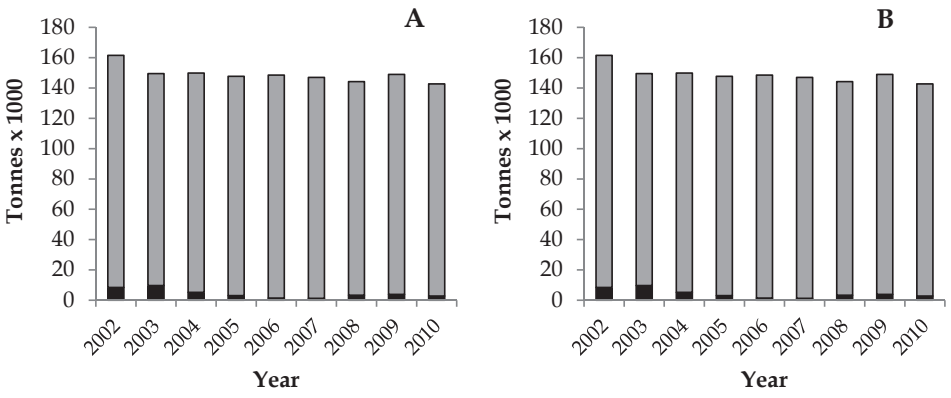

FIGURE 1 Collection and utilization rates of at-source separated municipal biowaste (A) and municipal wastewater sewage sludges (B) in 2002–2010 in Finland (Statistics Finland 2012).

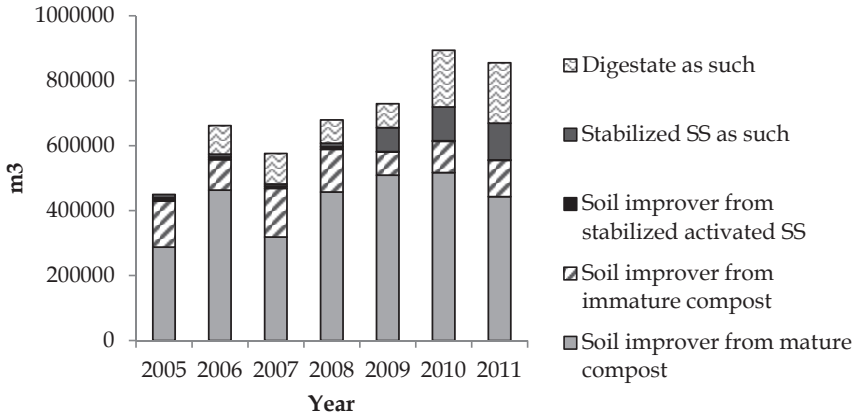

FIGURE 2 Production rates of soil improvers from biowaste and sewage sludges in 2005–2011 in Finland (Anon. 2012).

1.1.1 Modern era of composting industry

Knowledge of composting is evident from Biblical and medieval times (Haug 1993). Composting technologies had already been described with technical details by the Knights Templar in 1200 AD (de Bertoldi and Civilini 2006). For a long time composting was a simple process that was carried out on a small scale in the farmyard or back yard. The situation started to change in the 1930's–1950's, when research and experimental composting of different feedstocks including agricultural wastes, manures and night soil was carried out in

different parts of the world. In the 1940's–1960's a number of technological solutions for aerobic stabilization of waste were patented and pilot plants constructed in the US and Europe (Diaz and de Bertoldi 2007). In the 1980's the Commission of European Communities has been active in the waste management sector on the issues of recycling, composting, and energy recovery and financed a number of research programs that served as a catalyst for further boosting of the industry. During next 20 years a large number of composting plants was build all over Europe (de Bertoldi *et al.* 2000). After a promising start, however, many composting plants had to be closed and the planning of new ones delayed due to problems associated with a lack of odour nuisance control, poor quality of compost due to the presence of inert materials, heavy metals, and instability of compost. After the implementation of an at-source separation system the situation has improved significantly. By the year 2000 there were already 600 composting plants in Germany, about 300 in France, 70 in Italy, and 30 in Spain. Most of them produced very high quality compost utilized in plant nurseries, greenhouses, orchards and horticulture (de Bertoldi *et al.* 2000). In 2009, there were about 2500 sites in Europe for the composting of source segregated materials with an annual capacity of 27 million tons and an estimated annual capacity increase of 0.5 to 1 million tons. Additionally, there were 800 small agricultural co-composting plants, mainly in Germany and Austria (Anon. 2012b). In the USA similar problems of malodours and poor quality of compost were met being the reason for closing almost half of the facilities constructed in the US between 1980's and 2000´s with only 12 remained (Yepsen 2009).

In Finland composting of municipal sewage sludge started in 1970's. The first reactor composting plant for biowaste was built in 1992 and construction of similar plants started in 1995 all over the country (Lehto and Ekholm 2001). By 2005 the number of reactor composting plants was 15 and open windrow plants 18 with a total annual treatment capacity of 231 600 tons (Huhtinen *et al.* 2007). The main problems of compost plants in Finland were odour nuisance due to a short retention time in the reactors and an underestimation of plant capacity in relation to the amount of biowaste that the plants received for treatment. Therefore, by the year 2010 the capacity of composting plants was increased to 300 000 tons. The problems of poor quality due to heavy metals and inert materials that were met in other countries were avoided as an at-source separation system for biowaste was implemented in parallel with the construction of the composting capacity.

Over the last decade the image of composting in Europe has improved significantly and the market for compost products has been slowly growing in many EU countries. However, the situation is rather fragile; therefore, in order to keep the demand in the market stable, a high quality product should be produced. In 2010 ECN launched a quality assurance scheme (ECN-QAS) that aims to establish quality standards for compost as a product, needed for a cross-border movement of goods in the EU (Anon. 2010b). Additionally, end-of-waste criteria for biodegradable waste are under development in the European

Commission that will lay a legal base for the point when compost ceases to be waste and can gain product status (Anon. 2012b).

1.2 Main aspects of the composting process

1.2.1 Active and maturation phases

Composting is biological decomposition and transformation of organic matter (OM) under controlled, aerobic conditions that lead to formation of a humus-like stable product, which can be beneficially applied to land and plant production (Epstein 1997).

The process can be divided into active and maturation phases (Fig. 3). During the active phase readily degradable compounds are mineralized and metabolized into CO_2, H_2O, NH_3, NO_3-N, VOCs, and organic acids (Bernal *et al.* 2009). Rapid mineralization also leads to release of heat and development of thermophilic temperatures (> 45 °C) (Epstein 1997). The active phase can be additionally divided into three sub-phases: (i) mesophilic I (10–42 °C), (ii) thermophilic (45–70 °C) and (iii) mesophilic II (Keener *et al.* 2000, Ryckeboer *et al.* 2003b). Mesophilic sub-phase I is characterized by temperature rise that may continue from only a few hours up to a couple of weeks depending on the volume of the composted mass and climatic conditions. The thermophilic sub-phase may last for a few days, several weeks or even months and is critical for destruction of pathogenic micro-organisms. During mesophilic sub-phase II the temperature gradually decreases to ambient levels, which may take several weeks or months. The active phase is characterized with high O_2-uptake and CO_2-release rates, and high odour potential (Haug 1993). After rapid mineralization, composting moves into a maturation phase, when more rigid compounds are degraded or utilized by microorganisms. The maturation phase is characterized by low O_2-uptake and CO_2-release rates, low odour potential and temperatures close to the ambient values (Haug 1993). Stabilization of organic matter due to humification processes also occurs during this stage. Mature compost of high quality is rich in humus and can be used as soil conditioner or organic fertilizer.

1.2.2 Microbial succession during composting

Degradation of OM occurs under natural conditions in soils, sediments or in water bodies. Due to dispersal of the degrading material, it takes place at ambient temperature. Composting, in contrast, is characterized by abundance of easily degradable substrate, high densities of microorganisms and high metabolic activities that result in significant temperature rise (Insam and de Bertoldi 2007). The constant change in the surrounding conditions (T, pH, aeration, moisture, availability of substrates, etc.) defines succession in microbial populations, when exponential growth and stationary phases of one

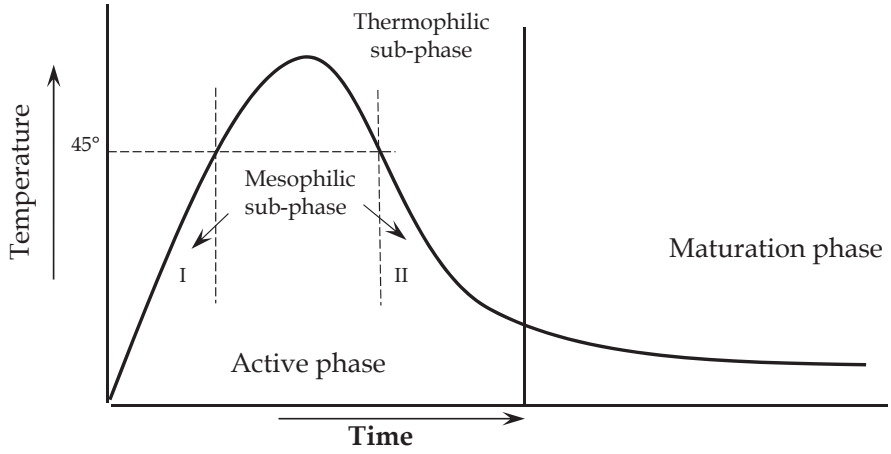

FIGURE 3 Phases of composting related to temperature dynamics.

group is changed by another (Fig. 4). Composting may be regarded as a sequence of continuous cultures, each of them with their own physical (e.g. temperature), chemical (the available substrate), and biological (e.g. the microbial community composition) properties and feedback effects (Insam and de Bertoldi 2007). Due to succession of microbial communities OM degrades in a rather short period of time.

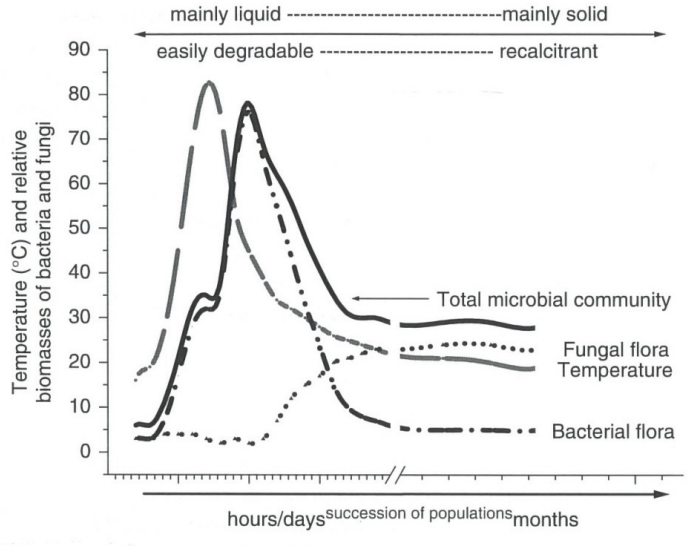

FIGURE 4 Microbial communities during the composting process: temperature feedback (Insam and de Bertoldi 2007).

The microorganisms present at the beginning of the process are introduced with the feedstock. Input materials are very heterogeneous and rich in microorganisms (Klammer *et al.* 2008), therefore, data on the microbial composition of the feedstock are not systematic. For example, bacteria strains found in the source-separated biowaste were *Lactobacillus* spp., *Leuconostoc, Pseudomonas, Acetobacter* spp., *Actinobacteria* and *Bacillus* spp. (Partanen *et al.* 2010), in fruit and vegetable waste *Bacillus, Lysinibacillus, Lysobacter, Staphylococcus, Enterobacter, Pseudomonas* and *Serratia* (Hayat *et al.* 2012).

During the first mesophilic stage the microbial biomass significantly increases, reaching densities up to 10^{12} cells g^{-1} (Insam and de Bertoldi 2007). Three to six-fold increase from the initial numbers of total microorganism and plate counts was observed on this stage (Klammer and Baath 1998, Narihiro *et al.* 2004). The most frequent in number are bacteria, actinobacteria, fungi and yeast that are generally referred to as primary decomposers (Ryckeboer *et al.* 2003b, Insam and de Bertoldi 2007, Fuchs 2010, Insam *et al.* 2010). They degrade energy-rich, readily biodegradable compounds, like sugars and proteins, excrete metabolic product and create physico-chemical conditions suited for secondary organisms, which cannot attack the initial substrates, but can degrade the excreted metabolites. It has been demonstrated that the number of mesophilic organisms in the original substrate is three orders of magnitude higher than the number of thermophilic organisms, but the activity of the primary decomposers induces the temperature rise (Insam and de Bertoldi 2007). Fungi compete with bacteria for the easily available substrate. Since the maximum specific growth rates of bacteria exceed those of fungi by one order of magnitude, fungi are very soon outcompeted. Bacteria found in this phase were *Acinetobacter* sp., *Bacillus* spp., *Cellulomonas* spp., *Klebsiella* spp., *Pseudomonas* spp., *Streptomyces* spp. and many others (Ryckeboer *et al.* 2003b).

In the thermophilic stage, organisms adapted to higher temperatures gain a competitive advantage and gradually, almost entirely replace the mesophilic flora (Ryckeboer *et al.* 2003a). Previously flourishing mesophilic organisms die off and are eventually degraded by the succeeding thermophilic organisms, along with the remaining, easily degradable substrate (Insam and de Bertoldi 2007). Thermotolerant and thermophilic bacteria and actinobacteria are known to remain active also at high temperatures. Thermophilic fungi that have growth maxima between 35 and 55 °C are also active before the establishment of higher temperatures that ceases the fungal growth (Thambirajah *et al.* 1995, Ryckeboer *et al.* 2003b). Temperature range from 50 to 65 °C is of the selective advantage, particularly for the genus *Bacillus.* Also, members of the group *Thermus* dominate on this stage, reaching numbers as high as 10^7 to 10^{10} per gram of dry weight in biowaste compost (Ryckeboer *et al.* 2003b). Thermophilic stage is critical in destruction of large number of human, animal and plant pathogenic organisms of all groups, including bacteria, viruses, fungi and parasites (Böhm 2007).

When the activity of the thermophilic organisms ceases due to exhaustion of the substrates, the temperature starts to decrease that leads to mesophilic

stage II. Mesophilic organisms recolonize the substrate, either originating from survived spores, through spread from the protected micro-niches, or from the external inoculation. The dominating organisms on this stage are bacteria and fungi that are able to degrade starch or cellulose (Insam and de Bertoldi 2007). The humification processes start also at this stage and continue during the maturation phase. During this phase, the number of bacteria decreases, but their diversity increases. At the same time, the population of fungi grows in quantity and in diversity, thus, fungal activity gaining the main importance during the maturation phase (Klammer and Baath 1998, Ryckeboer *et al.* 2003a). Compounds that are not further degradable, such as lignin-humus complexes, become predominant in mature compost.

1.2.3 Important parameters of composting

Compost is the product generated by the biological process (Haug 1993). Therefore, the quality of compost as a product depends on each constituent of the process such as the quality of the feedstock material (physical and chemical characteristics), the design of the active and maturation phases (open vs. reactor) and the operating conditions within the system (hygienization, temperature regulation, air supply, exhaust gas treatment, etc.). Failure at each stage of the process may lead to poor quality compost or even shutting down of the composting facility due to odour nuisance or hygienization problems.

Feedstock properties and conditioning

Nowadays, a wide range of materials are commercially composted: kitchen biowaste, garden waste, sludges and digestates from wastewater treatment and biogas production, animal manures, and others. To obtain high quality compost, the main aspects of the feedstock that should be taken into consideration are absence of chemical contaminants and inert objects, optimized physical properties (particle size, texture, moisture) and balanced C/N ratio.

Absence of inert objects and at-source separation of hazardous organic xenobiotics and heavy metals is crucial because while composting proceeds the biodegradable compounds are decomposed and non-biodegradable constituents concentrate in the product, making it inappropriate and even dangerous for land application.

The particle size and texture of the composting material should provide porosity to the mass in order to avoid an excessive compaction of solid material and to permit air to pass through the substrate. A structurally weak material would compact when piled and collapse when moistened (de Bertoldi *et al.* 2000). Additionally, degradation speed is directly proportional to the total surface area exposed to the microbial attack; the smaller the particles, the greater the ratio of surface area to the mass (Bernal *et al.* 2009). If particles are extremely large, decomposition rates and heat output will be low which will increase composting time and prevent thermophilic temperatures being reached (Keener *et al.* 2000). Optimization of composting mass texture is reached by using an adequate amount of bulking agent.

Moisture is a crucial aspect for microbial activity as decomposition by microorganisms occurs predominantly in the thin films (biofilms) on the surface of the organic particles (Ryckeboer *et al.* 2003b). The optimum water content of the compost mixture is 30–60 % (Keener *et al.* 2000, McFarland 2000, Gajalakshmi and Abbasi 2008). When the moisture content exceeds 60 %, penetration of the air becomes difficult and the process tends to become anoxic (Keener *et al.* 2000), while at moisture content below 30 % microbial activity critically decreases and the microorganisms become dormant (Ryckeboer *et al.* 2003a).

The optimal C/N ratio is between 25 and 35 (Keener *et al.* 2000). High C/N ratio makes initialization of the process very slow as microbes need N for their metabolism. In turn, at low C/N ratio there is an excess of N that may lead to formation of high, even phytotoxic, amounts of ammonia (Bernal *et al.* 2009). During the process the C/N ratio decreases as part of the C is lost as CO_2 due to microbial respiration while N is recycled into microbial biomass or stripped as ammonia (Ryckeboer *et al.* 2003b).

Optimisation of the C/N ratio and physical characteristics is usually achieved through mixing different types of recycled materials, the addition of bulking agents and organic or inorganic amendments (Haug 1993). The degree of organic matter exhaustion, i.e. amount of easily biodegradable material left after the pre-treatment of the feedstock, defines the oxygen demand for the system. However, this aspect is not much discussed in the scientific literature, although it is important for tuning of technology in each separate case.

Temperature

A rise in temperature at the beginning of composting indicates the progress of the process. 'Self-heating' is the result of organic matter degradation by aerobic microorganisms that carry out exergonic metabolism. Part of the energy is utilized by microorganisms to synthesise ATP while up to 50% is lost as heat (de Bertoldi and Civilini 2006). For example, full oxidation of glucose into CO_2 and O_2 leads to the release of 2815 kJ $mole^{-1}$ heat (Haug 1993), while fermentation to lactate releases 198 kJ $mole^{-1}$ heat (Madigan *et al.* 1997).

The optimal temperature range for microbial growth during composting is considered to be 40–65 °C (Keener *et al.* 2000, Bernal *et al.* 2009). At temperatures above 60 °C, the optimum for most thermophiles, the system starts to limit itself due to inhibitory high temperatures as mesophilic organisms are killed or become dormant (Ryckeboer *et al.* 2003b). Additionally, heat may limit O_2 supply to the microorganisms because it's solubility in water decreases with the increase of the temperature. However, a temperature above 55 °C is needed to kill pathogenic bacteria and is required by authorities for the hygienic control of the process. According to EU regulative acts 142/2011, 1069/2009 and 97/78/EY regarding treatment of animal by-products, hygienization should be carried out at min. 70 °C for 1h at particle size max. 12 mm or, alternatively, at 55 °C for min. 14 days. So, temperature rise is an essential part of the process and safety of the final product.

Oxygen and aeration

By definition, sufficient aerobic conditions define the nature of the process. With proper aeration O_2 is provided for the biological processes, temperature is controlled and excess moisture and CO_2 is removed (Haug 1993, Bernal *et al.* 2009). The stoichiometric demand for O_2 varies from a low of about 1.0 g g^{-1} OM for highly oxygenated substrates such as starch and cellulose to a high amount of about 4.0 g g^{-1} OM for saturated hydrocarbons, however, the amount is also dependant on the biodegradability of the substrates. Air requirements for drying and temperature control are usually much greater than the requirement for biological oxidation (Haug 1993). Although all the experts and compost plant operators agree on the necessity of O_2 for the process, recommendations for the optimum concentrations vary significantly, e.g. >> 5 % (Keener *et al.* 2000), > 10 % (Vallini *et al.* 2002), 5–15 % (de Bertoldi *et al.* 1993) or 15–20 % (Miller 1992). Insufficient aeration leads to the development of hypoxic conditions and noxious odours (Michel 1999, Vallini *et al.* 2002). In practice the main parameter used for defining the O_2 demand in the process is generation of foul odours. Among other parameters that define O_2 demand are stability and biodegradability of feedstock, stage of composting process, and technical design of the system. For example, highly unstable and biodegradable feedstock like biowaste requires more oxygen than more stable and slowly biodegradable park waste, or consumption of O_2 is higher during active stage and decreases as compost matures, forced-aerated solutions can deliver more oxygen to the system than passively aerated systems. Optimisation of O_2 supply is usually done on the stage of testing runs of the facility. At many composting plants operating in Finland, level of O_2 in reactors is set between 15 % and 18 % and during maturation stage windrows are passively aerated.

On the other hand, availability of O_2 to the microorganisms, but not the intensity of the aeration, is crucial. Degradation happens on the surface of the organic particles and O_2 should be supplied to the void pores between them. If hypoxic conditions are established, stronger aeration usually helps to solve the problem, but not necessarily. The problem of the excess aeration is quite common at the composting plants and lead to i) over-cooling of the system, ii) drying off the substrate due to the excess evaporation, iii) stripping out of metabolic products causing problems of malodours, and iv) channelling of the substrate, especially when air is blown through the substrate. Strong blowing of air causes formation of channels in the mass and, if too strong air flow is applied, air suction from the smaller adjoining channels may occur, creating local hypoxic areas on the microsites of the substrate (Hänninen and Himanen 2010). In addition, it is economically inefficient.

Providing sufficient aeration is the main aim of composting technology. However, its implementation in practice is difficult. Effective delivery of sufficient O_2 to the microsites of the substrate has been and still is the key challenge for operators and designers of composting plants.

Additives

Compost additives are mixtures of different amounts of various microorganisms, mineral nutrients or readily available forms of carbon, enzymes, and pH-balancing compounds that, according to trademark producers, are meant to enhance microbial activity, improve the composting process and the quality of compost.

In scientific literature, data on the effectiveness of compost additives is scarce. Razvi and Kramer (1996) presented the results of experiments, where they studied the effectiveness of seven commercial activators, top soil, and mature compost on the composting of grass clippings. They showed that commercial activators were not more effective than naturally available top soil or mature compost. Korhonen (2006) studied five accelerators commercially available in Finland by testing their effectiveness in composting a mixture of standard waste (potato 80 %, bread 15 %, and chicken feed 5 %) with wood waste as bulking agent (1/1.5, v/v) and showed that none had a positive effect on temperature, nutrient dynamics or phytotoxicity. On the other hand, some positive results were found for the addition of alkaline products to compost, e.g. ash or lime. Lau *et al.* (2001) reported that the addition of 10 % of fly ash had a positive effect on seed germination and reduced the availability of heavy metals. However, it increased electrical conductivity and volatilization of ammonia, while decreasing the bioavailability of phosphorous. Koivula *et al.* (2004) studied the effect of bottom ash on the composting of source-separated catering waste and found that a 10 % and 20 % ash addition improved the temperature regime, mineralization and humification rates, and decreased the loss of total nitrogen. Out of three lime addition rates to sewage sludge (0.63 %, 1 %, and 1.63 % dm) tested by Wong and Fang (2000) the most efficient was 0.63 %. This amount had a positive effect on composting by increasing temperature and CO_2 evolution without any negative effects on the microbial community.

1.2.4 Stability and maturity

The principal requirements for the application of compost as a product are stability and maturity. Both characteristics are defined in many ways and, usually, compost stability is related to microbial activity, while maturity is associated with phytotoxicity.

Stability is the extent to which organic matter has decomposed (Bernal *et al.* 2009). Epstein (1997) specified it as a stage in OM decomposition and as a function of microbiological activity. According to Iannotti *et al.* (1993) stability refers to the degree of OM decomposition and it is an indicator of the potential for microbial growth on the substrate. Stability is estimated by measuring indicators of biological activity per unit mass of compost. Methods include heat output in Dewar flask, CO_2 release, respirometry (Keener *et al.* 2000) and O_2 uptake in aqueous compost extract (Lasaridi and Stentiford 1998). Stable compost represents climax stage of microbial succession and is characterized by

little changes in OM matter composition over time. Release of CO_2 is ceased and structural changes occur mostly due to humification processes.

Maturity is an organo-chemical condition which indicates the lack of phytotoxic organic acids (Epstein 1997). According to Iannotti *et al.* (1994) maturity is absence of negative plant responses due to high cellulose content, allelopathic chemicals, toxic concentrations of metabolites of anaerobic metabolism, high salt content or other possible factors. Bernal *et al*. (2009) defines maturity as the degree of completeness of composting that implies improved qualities resulted from ageing of a product. Maturity is not described by a single property and is best assessed by measuring two or more parameters. A list of maturity indices established for compost of different sources is presented in Bernal *et al.* (2009), which includes germination index, water soluble C/N ratio, NH_4-N/NO_3-N and others.

Many experts relate stability and maturity to each other. According to Zucconi *et al.* (1981) stability and maturity go hand in hand as phytotoxic compounds are produced by the microorganisms in unstable compost. Bernal *et al.* (2009) specifies that besides relative stability of organic matter, maturity also describes the impact of other compost properties, including physical, chemical and microbiological. Keener *et al.* (2000) consider stability as an indicator of maturity.

1.3 Composts as soil improver and fertilizer

Mature compost is a multifunctional soil improver. It is widely applied in agriculture, horticulture, nurseries, landscaping, etc. The application of compost usually improves physical, biological and chemical properties of soil (Epstein 1997). As a source of organic matter, compost improves the soil's physical structure (porosity, bulk density), aeration and soil strength through aggregation of soil particles, where organic matter serves as a binding material. It also increases water retention and water-holding capacity, so water becomes more easily available to plants even in dry periods. Compost also improves chemical properties such as cation exchange capacity, soil pH, and electrical conductivity (Epstein 1997). It also serves as a source of slowly released organic N, C, macro- and micronutrients for soil organisms and plants. Mature compost is commonly characterized as having 2 % N, 2 % P and 1 % K (4.6 % P_2O_5, 1.8 % K_2O), although the numbers are influenced by the starting mixture and degree of stabilization (Keener *et al.* 2000). When applied to soil, compost enhances microbial activity (Insam *et al.* 2010). A wide range of studies have shown the ability of compost to suppress plant diseases and to decrease the growth of plant pathogens (Hoitink *et al.* 1997, de Bertoldi 2010, Saadi *et al.* 2010), thus reducing the need for pesticides.

With application of compost for plant growth, the cycle of OM circulation closes in, thus provide sustainable resource management of organic waste and animal by-products. Due to positive properties of the product, composting has

lately gained attention as an effective method for decreasing greenhouse gas emissions due to its ability to bind carbon in the soil (sequestration), act as a substitute for mineral fertilizers and peat in plant growth media production (Anon. 2010c). So, mature compost of high quality is a valuable product with beneficial features both for plants and soils.

1.4 Phytotoxicity of immature compost

On occasion, negative effects on plant performance, such as injuries to growing plants or to germinating crops, have been reported after application of composts from municipal solid waste (Zucconi *et al.* 1981a, Brinton and Tränker 1999, Wilkinson *et al.* 2009).

Phytotoxicity can be attributed to the characteristics of the material such as high salinity (Zucconi and De Bertoldi 1987) or heavy metal content (Epstein 1997, Tiquia 2010) or be a transient stage that is associated with immaturity of compost (Zucconi *et al.* 1981b, DeVleeschauwer *et al.* 1982, Brinton 1998). The negative effect of immature compost can be expressed as the direct impact on plant roots, but also as an indirect impact through increased soil microbial activity or increased competition between plant roots and microorganisms for availability of nutrients and oxygen. When an unstable product is applied to soil, it continues to decompose rapidly which leads to the creation of hypoxic conditions (Brinton and Evans 2002). A low oxygen level in soil has a direct effect on oxygen availability to plant roots, but also changes heavy metal solubility and uptake of nutrients to plants (Epstein 1997).

As mentioned, phytotoxic compounds may be produced in the initial stages of decomposition and tend to be inactivated as the process proceeds. Compounds claimed to be phytotoxic in immature compost include ammonia (Wong *et al.* 1983, Tiquia *et al.* 1996, Tiquia 2010), phenolics (Lilja *et al.* 1996, Ortega *et al.* 1996), and ethylene oxide (Wong *et al.* 1983). However, most frequently the phytotoxicity of immature compost is associated with LWCA (Chandrasekaran and Yoshida 1973, Chanyasak *et al.* 1982, DeVleeschauwer *et al.* 1982, Manois *et al.* 1987, Brinton and Tränker 1999).

The plant reacts to the inhibitory environmental conditions by lowering its metabolic rate, and, subsequently, by building up resistance expressed as shoot growth reduction, limited defoliation and temporary stunting. A strict dose-response effect associated with the stunting due to partial root destruction was observed (Zucconi *et al.* 1981a). However, if the first contact between roots and organic matter is not lethal, the plant shows the capability to recover and thrive in soils enriched with organic matter (Zucconi *et al.* 1981a). Among the number of aspects related to compost phytotoxicity this work concentrates on the role of carboxylic acids in the phenomena.

1.5 Low-weight carboxylic acids (LWCA) in compost

1.5.1 Behavior of LWCA in compost

Although LWCA are claimed to be an indicator of incompletely performed composting, they are a part of natural microbial degradation processes and unavoidable in any aeration procedure. A simplified scheme of LWCA behaviour during composting is presented in Fig. 5.

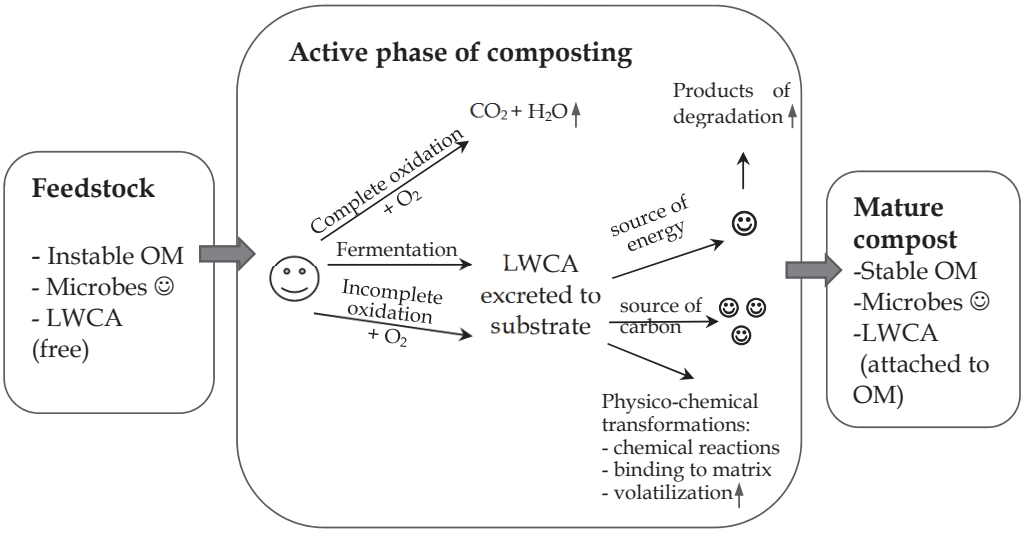

FIGURE 5 The scheme of LWCA behaviour during a composting process.

LWCA enter composting with the feedstock (Schuman and McCalla 1976, Sundberg *et al.* 2011), especially if it is piled for some time and oxygen becomes depleted during the storage. It is a common case in the waste treatment process that the at-source separated biowaste is stored first at the source of generation for several days and then at the composting plant before it enters the process. Digestate from anaerobic sludge of wastewater treatment is also rich in LWCA that are formed during digestion process.

LWCA are also formed during composting, especially during the early stage of the process. An abundance of easily degradable substrates in feedstock and the presence of different types of microorganisms boost the microbial activity. Microbes carry out OM degradation through different routes. As a result, a high amount of intermediate co-metabolites are formed, with LWCA among them. Although complete respiration is the only process that is aimed for during composting, simultaneous aerobic and anaerobic transformations happen constantly (Brinton 1998, Medina *et al.* 2009). Quite often the presence of

LWCA in compost is associated with dominating hypoxic conditions (Thompson *et al.* 2002).

The acids that enter composting with the feedstock as well as those formed during the process, serve as a source of carbon and energy for microbial biosynthesis (Schlegel 1986, Madigan *et al.* 1997). Due to these processes, concentration of LWCA decreases significantly during the active phase of composting.

In contrast to microbiological transformations of LWCA, that have been studied relatively well, physical and chemical behavior during composting is poorly presented. The primary aspect that has gained attention is volatilization of LWCA from compost due to the formation of foul odors. However, chemical reactions like esterification, association to the substrate and solubility in the water phase may happen. A better understanding of physical and chemical transformations of LWCA may help to manage malodors during composting in a better way.

1.5.2 Biochemistry and bacteriology of LWCA formation and degradation

Microorganisms carry out degradation of OM in two major biochemical pathways: 1) fermentation, in which oxidative-reductive processes occur in the absence of any added terminal electron acceptors and 2) respiration, in which molecular O_2 serves as the terminal electron acceptor. In relation to O_2 microorganisms are divided into aerobes that need O_2 for metabolism or growth and anaerobes that don't need it. Among aerobes there are groups that 1) require O_2 for metabolism and growth (obligate aerobes), 2) do not require, but grow better with O_2 (facultative aerobes) and 3) require, but at levels lower than atmospheric (microaerophillic aerobes). For anaerobes oxygen may be harmful (obligate anaerobes) or not required for growth (aerotolerant anaerobes) (Madigan *et al.* 1997). In compost strict aerobes are found in abundant amounts. However, facultative aerobes, obligate and aerotolerant anaerobes are active during composting, too (Medina *et al.* 2009). Almost one per cent of the bacteria number found in municipal solid waste compost was anaerobic and, from mesophiles, aerotolerant anaerobes were primarily active (Atkinson *et al.* 1996). Diaz-Raviña et al. (1989) reported 10^2 to 10^4 anaerobic cellulolytic bacteria per gram of dry solids compared to 10^4 to 10^6 aerobic cellulolytics in four composted urban refuse.

Out of the five sub-groups of microorganisms only obligate aerobes carry out complete oxidation of the substrate into CO_2 and H_2O. Microorganisms that belong to other groups conduct preferably fermentation with release of a big variety of end-products including alcohols, sugars, acids, aldehydes, ketones, etc. There is even a small groups of suboxidant aerobes, like some acetic acid or lactic acid bacteria, that carry out incomplete oxidation excreting acetate, lactate, keto- and oxyacids in the presence of O_2 (Schlegel 1986, Madigan *et al.* 1997). LWCA are products of many specific fermentation pathways that lead to excretion of particular acids in the extracellular substrate (Fig. 6).

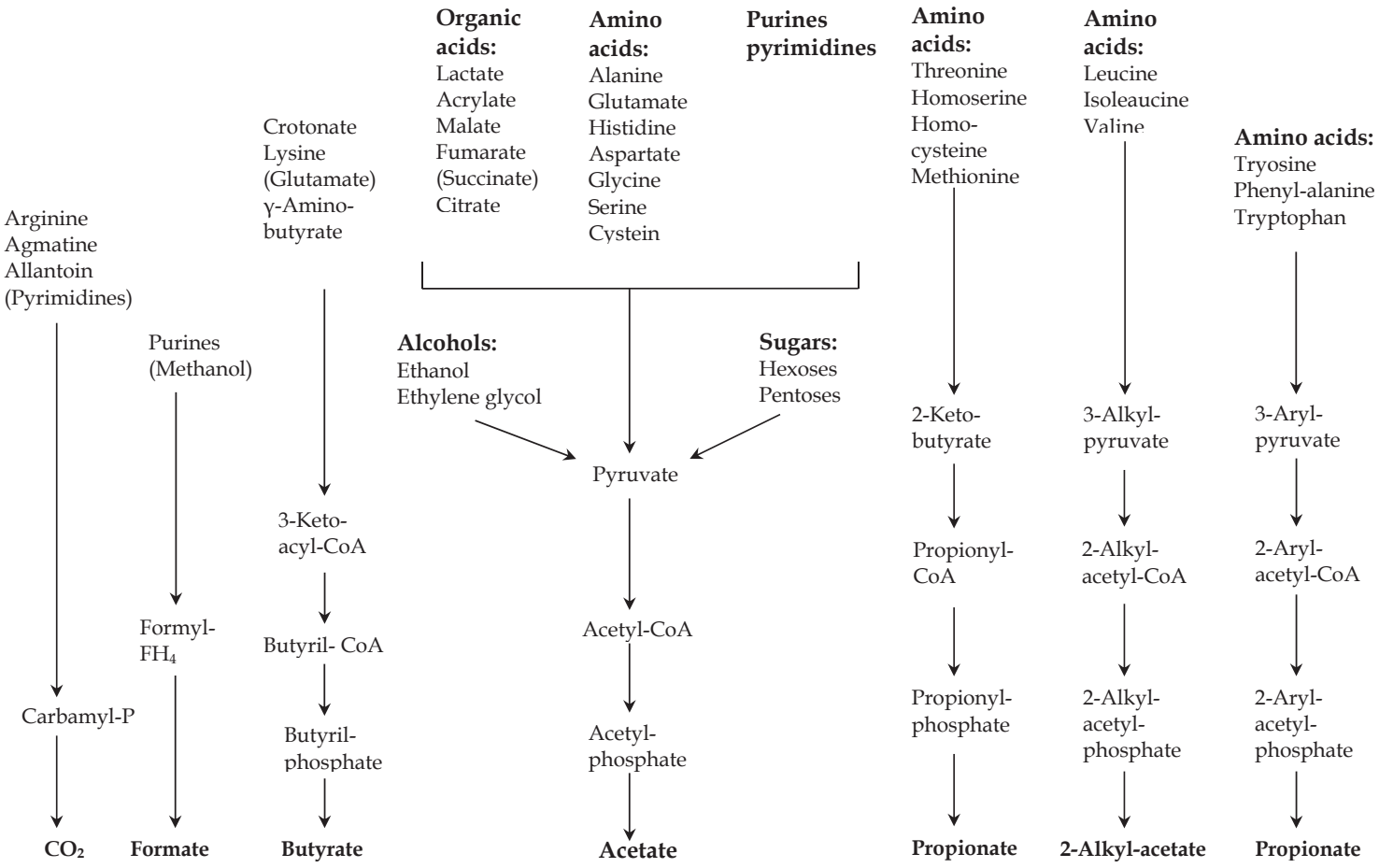

FIGURE 6 Pathways for the anoxic breakdown of various fermentable substances (Madigan *et al.* 1997).

Specific acid fermentations relative to this study with the organisms carrying them out are: homoacetogenic fermentation (*Acetobacter, Gluconobacter*), propionic acid fermentation (*Clostridium propionicum*), butyric acid fermentation (*Clostridium butyricum*), caproic acid fermentation (*Clostridium kluyveri, aceticum*). Additionally, there is a mixed acid pathway (*Escherichia, Salmonella, Shigella, Klebsiella, Enterobacter*), where lactate, acetate and formate are produced (Madigan *et al.* 1997). In many fermentation pathways acetate is formed as co-metabolite in addition to main products.

Besides terminate products of degradation, LWCA serve as a carbon source for many microorganisms. For example, bacteria can assimilate acetic acid by inducing enzymes of the glyoxylate pathway, which is based on the function of isocitrate lyase and malate synthase (Schlegel 1986). Some bacteria are also able to utilize propionic acid through two possible routes, either α-oxidation to pyruvate or via hydroxiglutarate (Cherrington *et al.* 1991). In syntrophic communities, one LWCA can be utilized as substrate and another can be excreted as end-product (Jackson and McInerney 2002). It was demonstrated that degradation of propionate is carried out with the interspecies transfer of formate by synthrophic propionate-oxidizing bacteria from the class Deltaproteobacteria (Dong and Stams 1995). Bacteria of the genus *Syntrophomonas*, *Thermosyntropha lipolytica* and *Synthrophothermus lipocalidus* are capable of synthropic butyrate metabolism (Plugge *et al.* 2010). Although syntrophic communities have been mainly studied in methanogenic environments, their presence in compost has been demonstrated too (Rhee *et al.* 2002).

1.5.3 Physico-chemical characteristics of LWCA

During composting, in addition to microbiological transformations, physical and chemical changes of LWCA occur as well. Therefore, the physico-chemical properties of the compounds play important role as they define the fate of the compounds in the compost mass, including volatilization and adsorption to the matrix. Table 1 presents selected physico-chemical characteristics of C_1–C_6 LWCA.

With the increase in carbon chain length, solubility of the LWCA in liquid-water and gas-water phase decreases, meaning that the acids with longer chains tend to reside mainly in the gaseous phase or are easily attracted to lipophilic components in the compost mass. As carbon chain length grows, boiling point increases, which should be taken into consideration in analytical procedures. Odour detection threshold decreases by several orders of magnitude from formic to valeric acid.

LWCA are monocarboxylic acids with a common structure of the functional carboxylic group (R–COOH) that dissociates in water into carboxyl anion ($R–COO^-$) and hydronium cation (H_3O^+):

$$R–COOH + H_2O \rightleftarrows R–COO^- + H_3O^+$$

TABLE 1 Physico-chemical properties of the studied LWCA: molecular mass (M), dissociation constant (pK_a), solubility in water (liquid-liquid phase, $S_{l\text{-}l}$), octanol/water partitional coefficient (log P_{ow}), Henry's law constant (K_H), solubility for gas-liquid phase ($S_{g\text{-}l}$), boiling point (BP) and range of odour detection threshold for human (ODT).

Acid	M g mol^{-1}	pK_a[a]	$S_{l\text{-}l}$[b] mg l^{-1}	log P_{ow}[b] log K_{ow}	K_H[c] atm-m^3 mole^{-3}	$S_{g\text{-}l}$[c] mol kg^{-1}	BP[d] °C	ODT[e] mg m^{-3}
Formic	46.03	3.737	1x10^6	–0.54	1.81x10^{-7}	1.38x10^{-4}	101	2–640
Acetic	60.05	4.757	1x10^6	–0.17	1.81x10^{-7}	1.56x10^{-5}	117.9	0.025–10
Propionic	74.08	4.875	1x10^6	0.33	1.77x10^{-7}	1.31x10^{-5}	141.5	0.003–0.89
Butyric	88.11	4.83	6x10^4	0.79	2.20x10^{-7}	1.14x10^{-5}	163.8	0.0004–42
Valeric	102.13	4.763	2.4x10^4	1.39	4.72x10^{-7}	5.30x10^{-6}	186.1	0.0008–0.12
Carpoic	116.16	4.800	1.0x10^4	1.92	7.58x10^{-7}	3.0x10^{-6}	205.2	0.02–0.52

[a] at 25 °C, Serjeant and Dempsey (1979), [b] at 25 °C, SRC (2011), [c] K_H at 298.15 °K and $S_{g\text{-}l}$ at *p*HA=10^{-10} atm and pH=5, Khan and Brimblecombe (1992), [d] at outside pressure of 760 mmHg, Lide (2004), [e] O'Neill and Phillips (1992).

The degree of dissociation is dependent on the pK_a of the acid and the pH of the solution, and can be predicted using the Henderson-Hasselbalch equation:

$$pH = pK_a + \log_{10} [R\text{–}COO^-]/[\ R\text{–}COOH]$$

As undissociated forms of the acids are volatile, volatilization of LWCA from compost is more extensive at low pH. In some sources LWCA are even referred to as volatile fatty acids (VFA) as they can be steam-distilled under acidic conditions (Thompson *et al.* 2002).

1.6 Analytical methods for quantification of LWCA

Analysis of LWCA in solid compost substrate is usually divided into two steps: 1) extraction or distillation of LWCA from the solid phase into liquid and 2) determination of the extracted LWCA in the solution. Extraction of the LWCA from compost was done with phosphate buffer at pH 7 (Thompson *et al.* 2002), ether (Manois *et al.* 1987), water at neutral (DeVleeschauwer *et al.* 1982) or acidic pH (Manois *et al.* 1987, Brinton and Tränker 1999).

Quantification of different types of acids in the liquid phase is possible if a separation technique is applied. High pressure liquid chromatography (HPLC)is used to some extent for LWCA analytical separation (Brinton 1998, Robertsson 2002). The most popular LC detectors are based on refractive index (RI), ultraviolet (UV) and conductivity (CD) measurements. A major problem with detection of LWCA is that the chromatographic properties of carboxyl groups are weak, resulting in relatively low sensitivity and selectivity of detection (Käkölä 2009). Gas chromatography (GC) is a very effective and widely applied method for LWCA separation (Alén *et al.* 1985, Thompson *et al.* 2002). Quite often a flame-ionization detector (FID) in combination with GC is

used for LWCA detection. Analysis of LWCA using GC-FID was conducted in many key studies on LWCA dynamics during composting and phytotoxicity (Lynch 1977, DeVleeschauwer *et al.* 1982, Manois *et al.* 1987). The method allows detection of free LWCA molecules, starting from acetic acid onwards, i.e. C2–C6 because FID does not respond to formic acid due to the small size of the molecule (Thompson *et al.* 2002). Therefore, data on the presence and dynamics of formic acid in composting is scarce. Quantification of the formic acid becomes possible if esterification, chemical derivatization, or both are applied to enlarge the size of the molecules.

2 OBJECTIVES

The main aim of this thesis was to study phytotoxicity of composts at different stages of the process and to evaluate the role of low-weight carboxylic acids in phytotoxicity. The evaluation was done based on the data presented in the original publications (I–V) and described in this summary (experimental series 2). The specific objectives of the study were the following:

- Develop an analytical method for quantification of LWCA in composts, formic acid in particular, by applying a derivatization technique for GC-FID detection (I–III).
- Evaluate effects of different parameters (feedstock, aeration, additives) on the composting process, formation of LWCA and phytotoxicity (I, II, experimental series 2).
- Obtain phytotoxically effective concentration values for the individual LWCA and assess the type of LWCA phytotoxicity in mixtures (IV–V).
- Evaluate role of the LWCA mixtures in the phytotoxicity of composts (I, II, IV, V).

3 MATERIALS AND METHODS

3.1 Setup of the composting experiments

Three series of composting experiments were conducted for studying the effects of feedstock, aeration and commercial mineral-based additives on the process and the quality of compost, paying special attention to the dynamics of LWCA and phytotoxicity.

3.1.1 Experimental series 1: Effect of feedstock (I)

Laboratory experiments were performed with three different feedstocks – at-source separated kitchen biowaste (BW), aerobic municipal sludge (AS), and dewatered municipal anaerobic sludge (AnS). Each feedstock was mixed with peat as the bulk material in proportion 1/1 (v/v). Details on the origin of the feedstocks and the bulk material, as well as characteristics of the mixtures, are presented in the original publication (I). Insulated lid-covered composter (vol. 220 litres; Biolan, Finland) supplied with a leachate-collecting system was filled with one of the feedstock+peat mixtures (BW, AS or AnS), each feedstock having one treatment. The compost mass was aerated through a system of bored holes and was based on the air-pressure difference between the inner and outer parts of the composter. The experiment lasted for 63 weeks, during which time the process was monitored (see 3.1.4 for details), the composting mass was mixed manually, and samples were collected for further analysis.

3.1.2 Experimental series 2: Effect of aeration

Results of the experiments were published in conference proceedings (Himanen *et al.* 2007), but not in a peer-reviewed international source.

Laboratory trials with two aeration rates were carried out in insulated lid-covered composters (vol. 220 litres; Biolan, Finland). Two composters were filled with kitchen biowaste mixed with wood chips and sphagnum peat in proportions 6/3/1 (v/v/v). Kitchen biowaste was a mixture of food leftovers

and vegetable waste that contained fish and meat residues, vegetable stunts, cabbage leaves, carrot and potato peels, etc.). The waste was collected for three days at Mikkeli food catering centre (Finland). Wood chips and peat were collected at Mikkeli waste treatment facility. Forced aeration was applied for the first 16 weeks at the rates of 0.5 l min^{-1} (AR0.5) and 5 l min^{-1} (AR5), after which forced aeration was stopped and compost was matured with passive aeration. There was one treatment for each aeration rate. The trial lasted for 52 weeks, during which the process was monitored (see 3.1.4), the composting mass was mixed manually and samples were taken for further analysis.

3.1.3 Experimental series 3: Effect of compost additives (II)

Two commercially available mineral-based additives were studied in laboratory experiments in insulated lid-covered composters (vol. 220 litres; Biolan, Finland) (see 3.1.1 for details). According to the manufacturer of additive A, this is a mixture of zeolite or kaolin clay (70 %), Mn_2SO_4 (10 %), dolomite chalk (15 %), ashes (5 %), and Co_2SO_4 (< 0.3 %). The additive is sold under the name of GrowHow Komposti Eliksiiri (Kemira, Finland), formerly Biodeg, and is claimed to accelerate the composting process, quickly stabilize pH, and speed up the humification process. Additive B is a mixture of $Ca(OH)_2$ (> 90 %), CaO_2 (> 6 %) and CaO (~ 1 %) and is sold under commercial name of Velox (Nordkalk Oyj Apb). The additive is claimed to accelerate the composting process, remove malodours and disinfect waste.

For the experiments, three composters were filled with the following mixtures: at-source separated biowaste and peat in proportion 1/1 (v/v) (BW), biowaste + peat mixture and a clay-based additive in proportion 1 kg $(100\ kg)^{-1}$ biowaste (BW+A), and biowaste + peat mixture and calcium hydroxide based additive in proportion 1.75 kg $(100\ kg)^{-1}$ compost mass (BW+B). Each mixture had one treatment. Details on the origin of the feedstocks and bulk material, and characteristics of the mixtures are presented in the original publication (II). The experiments started in winter (January), therefore the biowaste was frozen. The experiments lasted for 52 weeks, during which the composting mass was monitored (see 3.1.4), mixed manually, and sampled for further analysis.

3.1.4 Monitoring of the experiments

The progress of composting was monitored by measuring the temperature of the compost mass at 50 cm depth and the concentrations of gases inside the composter above the mass using an infrared gas analyzer for oxygen, carbon dioxide and methane (GA94, Geotechnical Instruments) or Dräger detection tubes for gaseous ammonia (2/a and 5/a, National Dräger Inc, USA).

3.2 Chemical analyses

3.2.1 Dry matter, ash content and pH

Dry matter (dm) and ash content of the compost samples were measured according to the standard CEN 13039 (CEN 1999b). Analysis of pH was made according to the standard method CEN13038 (CEN 1999a, CEN 1999c).

3.2.2 Analysis of LWCA (I-III)

Development of the analytical method started with measuring the effectiveness of the alkaline extraction of LWCA from compost using 0.1 M NaOH. The extracted salts were further derivatized with methyl alcohol and the acid esters were analyzed with GC-FID (III). The method allowed quantification of 11 acids from formic (C_1) to capric (C_{10}) and isomers of butyric and valeric acids. External standard and standard addition techniques were used for quantification of LWCA. For the details of the method refer to the original publication (III). The method was shown to be effective and precise, however, too laborious for a serial analysis.

The suitability of an alternative method, developed by Alén et al. (1985) for LWCA analysis in alkaline pulping liquor, was evaluated. As in the first method (III), LWCA extraction was made with 0.1 M NaOH and additionally with water. Derivatization was carried out with benzyl bromide and LWCA derivatives of 8 acids (C_1–C_6) and isomers of butyric and valeric acids were analyzed using GC-FID. For LWCA quantification external standard of pure LWCA as well as crotonic acid as internal standard were used. For other details on the procedure refer to the original publications (I; Alén *et al.* 1985). The method was shown to be suitable for serial analysis of LWCA in the samples obtained in the composting experiments (I, II, and experiment 2). Concentrations of LWCA in compost are expressed as mg kg^{-1} dm.

3.3 Phytotoxicity assays (I-V)

3.3.1 General set-up for measuring phytotoxicity

Phytotoxicity of the compost samples was evaluated in short-term assays using an extract of compost or in subchronic assays using a mixture of compost and peat-based growth media (PBGM). Dose-response relationships of individual LWCA and evaluation of their mixture toxicity were also conducted in short-term assays on pure LWCA solutions or in subchronic assays, where the solutions were added to the solid substrate. Comparison of the toxicity potentials between individual acids was made based on the effective

concentration (EC) values extrapolated from the modelled data of dose-response assays.

3.3.2 Short-term assays

Phytotoxicity of the compost samples (I, II, experimental series 2)

The assay was developed on basis of a range of short-term compost phytotoxicity tests. Short-term phytotoxicity of composts was evaluated on compost – deionized water extracts (1/1, v/v). For the extract preparation 300 mL of compost was mixed with 300 mL of deionized water, shaken for 1 h at 180 rpm, centrifuged at 5000 rpm for 15 min, filtered through pre-rinsed filter paper (Whatman no. 4, Ø15 cm), divided into batches of 40 ml and frozen at –20 °C until needed. Five milliliters of the melted extract or de-ionized water as control were added to a Petri dish lined with filter paper. Twenty seeds of cress *Lepidium sativum* L. were placed in each dish and incubated in the darkness at 25–27 °C. After 48 h, the number of germinated seeds and the total length of the radicle (shoot + root; accuracy 1 mm) were measured. Each plate had three replicates and an extract from each compost sample was tested five times. For details and validation of the assays see the original publications (I, II). The compost was considered to be non-toxic, when the results of the *t*-test showed no statistically significant difference ($p > 0.05$) between the parameter (germination or seedling length) in the compost and control.

Short-term assays for EC values and mixture effect of LWCA (IV, V)

The first set of the assays for dose-response modeling was conducted on a series of seven dilutions of pure formic, acetic, propionic, butyric, valeric, or caproic acid (IV). The second set of the assays was conducted on a series of five dilutions of pure formic (F), acetic (A), or propionic (P) acid (V). Incubation conditions between the sets differed slightly from each other, therefore, the results are presented separately for each set. Data from the latter set were used for designing the assays for evaluation of the mixture effect of LWCA. De-ionized water was added to the control treatments.

Phytotoxicity of binary and ternary mixtures of LWCA was studied using the principle of concentration addition. Binary (F+A, F+P, and A+P) and ternary (F+A+P) mixtures were prepared using proportions of the respective EC50-values (= 1 toxic unit, TU). The EC50 concentrations were obtained in the preliminary individual toxicity assays. Summed assay concentrations of the binary mixtures were: ∑ 0.1 TU, ∑ 0.25 TU, ∑ 0.5 TU, ∑ 1 TU, ∑ 2 TU, ∑ 4 TU and in the ternary mixtures: ∑ 1/12 TU, ∑ 1/6 TU, ∑ 1/3 TU, ∑ 1½ TU, ∑ 3 TU.

Ten milliliters of the pure acid, acid mixture or control were added to a Petri dish lined with filter paper. Twenty seeds of cress were placed in a dish and incubated in the darkness at 24–26 °C for 72 hours. After incubation, number of the germinated seeds was counted and the total length of the radicle (root + shoot; accuracy 1 mm) was measured (IV, V). Results of the assays were

used for modeling dose-response relationships and evaluation of the mixture effect of LWCA.

3.3.3 Subchronic assays

Phytotoxicity of the compost samples (I, II, experimental series 2)

Subchronic assays were conducted on mixtures of the compost and peat-based growing medium (PBGM) at a 1/1 (v/v) ratio. PBGM (B2, Finnpeat SI400, Kekkilä, Finland) was a light sphagnum peat (von Post H1-3) based substrate, limed and fertilized, that was also used as a control. Plastic pots were filled with either PBGM-compost mixture or PBGM, and 25 seeds of cress (*L. sativum*) were spread on the surface and covered with a small amount of the PBGM. The pots were incubated for 21 days at a temperature of 25–27 °C, humidity 60% and 16/8 hours light/dark cycle. The plants were irrigated with deionized water on demand. On the day of termination, the germinated seeds were counted, plants were cut close to the substrate, dried overnight at 70 °C and weighted. Each sample had two replicates and a control with six replicates and the assay was repeated three times. Phytotoxicity of the compost was evaluated in the same way as in the short-term assays.

Subchronic assays for EC values and mixture effect analysis of LWCA (IV, V)

The assays were conducted according to ISO 11269-2 standard (ISO 2008). The first set of the assays for the dose-response modeling was conducted on a series of five dilutions of pure formic, acetic, propionic, butyric, valeric, or caproic acid (IV). The second set of the assays was conducted only for formic, acetic, or propionic acid (V) and data were used for designing the assays for evaluation of the mixture effect of LWCA. The growing conditions for the sets were slightly different from each other; therefore, the results are presented separately for each set. Experimental set I was conducted in an incubation chamber, whereas experimental set II was conducted in a greenhouse. Mixture toxicity of LWCA in subchronic assay was studied using the same principle and design as in the short-term assays.

For these trials, 0.1 l of sole acid, acid mixture, or control (deionized water) were added to 1.6 l of the inorganic growth substrate that was a mixture (6:1, v/v) of coarse-grained sand (particle size 0.5–1.2 mm; Maxit Oy, Finland) and quartz sand (particle size 0.2–0.005 mm; NFQ Nilsiän kvartsi, SP Minerals Oy, Finland). To prepare one experimental unit, a pre-rinsed plastic pot was filled as following: approx. one cm of peat layer on the bottom, 0.4 l of the inorganic substrate, 25 seeds evenly distributed, and approx. one cm of the peat on top. Peat layers were used to prevent leaching of the substrate from the pot during watering and to decrease stripping of the acids from the substrate. Each series of pots was held simultaneously for 21 days in a greenhouse at 21–29 °C (IV) or in the incubation chamber at 23–27 °C (V) with light/dark regime 16/8 hours, the light being 13 000 lx, 185 µmol m^{-2} s^{-1} and with the color temperature of 6500 K. The plants were irrigated on demand with the general fertilizing solution

(NPK 12-6-9, Kekkilä Kukkaravinne, Kekkilä Oy, Finland). On the day of termination amount of the germinated seeds were counted, plants cut close to the substrate, dried overnight at 70 °C and weighed. Results of the assays were used for modeling dose-response relationships and evaluation of the mixture effect of LWCA.

3.4 Data analysis

3.4.1 Dose-response modeling and calculations of the EC values

The results of the endpoints (germination, early seedling length and shoot biomass) obtained in the short-term and subchronic assays were used to model dose-response relationships for each acid and LWCA mixtures. Modelling, calculations of the EC values with their confidential intervals and estimation of statistical parameters were performed using R software (version 2.10.1) and the drc package. Out of the range of models tested, the three-parametric Weibull (IV) or the three-parametric log-logistic (V) were chosen as the non-linear models, which were used for the final data analysis. The mathematical expressions of the models can be found from the original publications (IV, V).

3.4.2 Toxicity analysis of LWCA mixtures

Toxicity analysis of the mixtures was made using the interaction index according to Marking (1985). For that, EC values and confidential intervals, obtained as described in 3.4.1, were used to calculate the sum of toxic action (S) and the additive index (AI) for each mixture. Based on the magnitude of S and 95 % confidence interval of AI, the type of interaction in LWCA mixtures was evaluated (V).

4 RESULTS

4.1 Composting experiments (I, II, experimental series 2)

4.1.1 Temperature dynamics

In all three experimental series the temperature dynamics were typical for a composting process (Fig. 7). In general, the thermophilic values (> 45 °C) were reached within the first week and lasted after that from 1 to 4 weeks. Depending on the feedstock and aeration rate, the active phase lasted from 3 to 13 weeks before the temperature reached ambient levels.

In the experiment series 1, composting of BW was more active compared to AnS and AS (I). Temperature peaks were observed twice, on week 1 (T_{max} = 57 °C) and 4 (T_{max} = 63 °C) and the active phase lasted for 9 weeks. In AnS thermophilic values were reached within 4 days (T_{max} = 57 °C) and the active phase was the longest, lasting for 13 weeks. By contrast, composting of AS proceeded at mesophilic temperature during the whole experiment (T_{max} = 44 °C) and the active phase was the shortest, lasting only for 5 weeks.

In the experiment series 2, mineralization of kitchen biowaste at an aeration rate of 5 l min^{-1} (AR5) was more intensive compared to compost aerated at 0.5 l min^{-1} (AR0.5) as the active phase was much shorter, 3 weeks (AR5) vs. 9 weeks (AR0.5). However, temperatures in AR 0.5 were significantly higher during the whole active phase and the maximum value was exceeded by 17 °C compared to AR5 (max. $T_{AR0.5}$ = 71 vs. T_{AR5} = 54 °C).

In the experiment series 3, in compost with additive A the compost temperature between weeks 2 and 3 was 5–10 °C higher than in the control or compost with additive B. In composts with additives the active phase appeared to be longer by one week compared to control, 6 weeks in BW vs. 7 weeks in BW+A and BW+B (II). Neither of the additives had an effect on the maximum temperatures which were about 60 °C in all 3 composts.

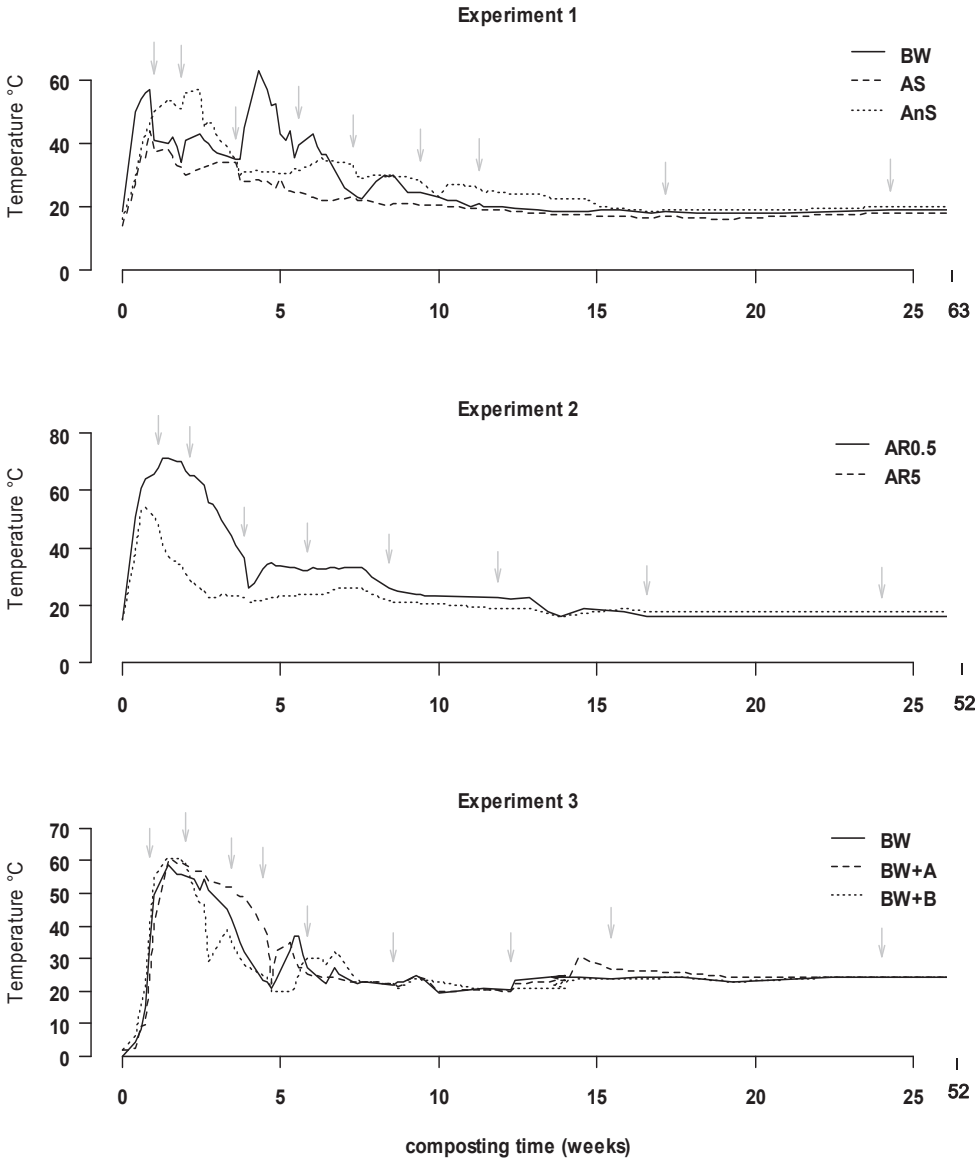

FIGURE 7 Dynamics of temperature in the composting experiments. Arrows indicate when mixing of the compost was done. Composted materials were: BW = kitchen biowaste/peat mixture (1/1, v/v), AS = aerobic sludge/peat mixture (1/1, v/v), AnS = anaerobic sludge/peat mixture (1/1, v/v), AR0.5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 0.5 l min^{-1}, AR5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 5 l min^{-1}, BW+A = biowaste/peat mixture (1/1, v/v) + clay-based additive A, BW+B = biowaste/peat mixture (1/1, v/v) + calcium-based additive B.

4.1.2 pH dynamics

In the experimental series 1 the dynamics of the pH in AS and AnS were similar to each other (Fig. 8). Composting of the sludges started from pH around 6 (AS = 5.7 and AnS = 6.4) followed by a rapid increase up to pH 7.6–7.8 during the first week and stayed on that level till week 4. Slow acidification was observed between weeks 4 and 16 when pH decreased as low as 3.5–3.8. However, it rose again to around 5 by week 63.

Use of peat as a bulking agent defined the initial pH values of the biowaste composts at acidic values. In all three experimental series, composting of biowaste started from pH 4.5–5 increasing slightly to basic values of 7–8.5 in the active phase and decreasing gradually to acidic values of 4.5–6.5 by the end of the series (Fig. 8). In the experimental series 2, in compost AR5 pH decreased from the basic values to neutral between weeks 4 and 6 and after that pH fluctuated around 6.5 till the end of the series. On the contrary, in compost AR0.5 gradual acidification happened between weeks 4 and 16, during which pH dropped as low as 5 and stayed on that level till the end of the series. Addition of the calcium-based additive B in the experimental series 3 led to an increase in the initial pH by 2 units (from 4.5 in BW to 6.4 in BW+B). After a peak of 8.7 on week 8, strong acidification was prevented and pH fluctuated around 7.1–7.8 till the end of the series. The dynamics of pH in compost with clay-based additive A did not differ significantly from the control, where pH increased from 4.5 to about 6 between weeks 2 and 4 and fluctuated around 6.5 till the end of the series.

Overall, a common pattern of increase in pH by 1–2 units from the initial values during the first 2 weeks of composting could be observed in all three experimental series. After that, slow acidification was registered, which lasted from 4 to 12 weeks. During this period, pH decreased either slightly, reaching neutral values of 6.5–7, or strongly, reaching acidic values of 3.5–4. However, after 52-63 weeks of composting pH had stabilized at slightly acidic or neutral values.

4.1.3 Dry matter content of compost samples

In the experimental series 1 dry matter content in BW compost was in the range of 25–40 % and in both sludge composts 15–30 %. In the experimental series 2 dry matter in AR0.5 ranged between 22 % and 37 % and in AR5 between 24 % and 30 %. In the experimental series 3 dry matter was about 40 % at the beginning of the experiments and gradually increased up to 55 % in BW, 64 % in BW+A and 48 % in BW+B by the end of week 52. Although initial values of dry matter were close to or lower than the optimal values, no free leachate was collected due to the high water holding capacity of peat.

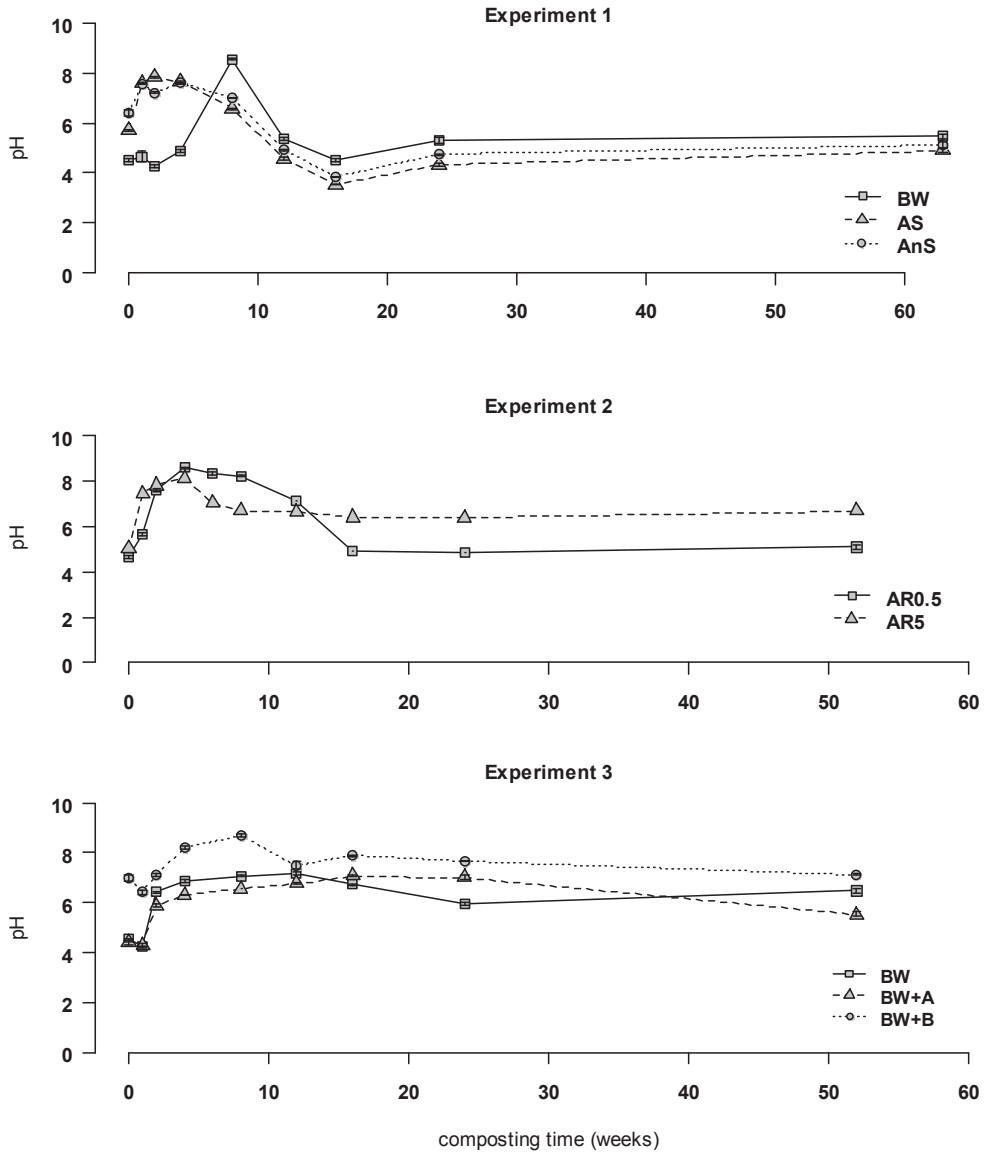

FIGURE 8 Effect of feedstock, aeration rate and additives on dynamics of pH in composting experiments. Composted materials were: BW = kitchen biowaste/peat mixture (1/1, v/v), AS = aerobic sludge/peat mixture (1/1, v/v), AnS = anaerobic sludge/peat mixture (1/1, v/v), AR0.5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 0.5 l min^{-1}, AR5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 5 l min^{-1}, BW+A = biowaste/peat mixture (1/1, v/v) + clay-based additive A, BW+B = biowaste/peat mixture (1/1, v/v) + calcium-based additive B. Error bars indicate standard deviations of the means of triplicates.

4.2 LWCA in composting process (I-III)

4.2.1 Analytical aspects of LWCA quantification in compost samples

Extraction of the LWCA with water or alkaline solution with further benzylation (I, II, experimental series 2) or methylation (III) was an effective pre-treatment method that allowed identification and quantification of the acids using GC-FID. The technique enabled quantification of formic acid in the presence of other LWCA. In spite of a high number of noise peaks produced by the compounds originating from compost or solvents, peaks of the derivitized esters of LWCA could be clearly identified. Examples of the chromatograms (Fig. 9 and Fig. 1 in III) exhibit narrow and sharp peaks that allowed effective identification and quantification. Among other LWCA, butanoic acid in compost samples was a challenge to identify, as the peak overlapped with the peaks of the other compounds. Analysis of several samples with gas chromatography-mass spectrometry (GC-MS) during development allowed verification of the retention time of the acid ester more precisely and helped identification in routine analysis.

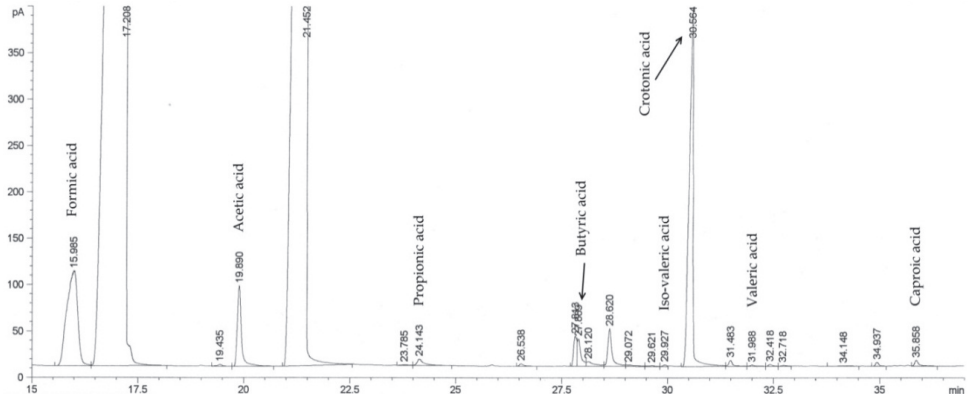

FIGURE 9 An example of the chromatogram with FID detection. The sample is the initial substrate of BW treatment used in the experimental series 3. LWCA were extracted with alkaline solution and derivitized with benzyl bromide.

For the method where LWCA were methylated, the detection limit for propionic, butyric, caproic, caprylic, and capric acids was 1 mg kg^{-1} dm; for formic, acetic, valeric, enanthoic, and pelargonic acids it was 5 mg kg^{-1} dm; and for *iso*-butyric acid it was 10 mg kg^{-1} dm (III). For the method used for the routine analysis, where LWCA were benzylated, the limit values were evaluated separately for each LWCA in every sample. On average LOD was 20 mg kg^{-1} dm and LOQ was 30 mg kg^{-1} dm.

In general, amounts of the total LWCA extracted with water were only 10 % to 50 % of the amounts extracted with alkaline solution (I-III). Extractability with water was higher for the propionic and butyric acids (30–100 %) compared to the formic and acetic acids (10–50 %). The exception was acetic acid in the beginning of experimental series 1 in all feedstocks and in experimental series 3 in BW+B, where it was almost 100 % water-extractable. In these cases the concentration of the acid exceeded concentrations of others by up to 10 times. Therefore, by using alkaline extractant it is possible to obtain a better picture of the concentrations of LWCA in compost.

4.2.2 Dynamics of the total concentrations of LWCA

Considerable amounts of LWCA entered the process with the feedstock. Initial total concentrations of LWCA extracted with alkaline solution measured in kitchen biowaste ranged from 4.0 to 6.0 g kg^{-1} dm and in municipal biowaste 12.3 g kg^{-1} dm (I, II). In sludges the total amount of LWCA in the beginning of the experiment was 14.3 g kg^{-1} dm in AS and 6.6 mg kg^{-1} dm in AnS (I). Application of calcium-based additive B, with calcium peroxide as an active component, tripled the initial concentration of LWCA, while clay-based additive A did not have a significant effect (II).

Concentrations of LWCA in sludge composts and biowaste with additive B have already dropped during the first week of composting to the levels which they fluctuated around till the end of the experimental series (Fig. 10). On the contrary, in biowaste compost only a slight decrease or even a net accumulation of LWCA was observed during the first 4 weeks of composting before the concentrations stabilized. Importantly, accumulation of LWCA in compost with stronger aeration (AR5) was higher than in compost aerated at lower rate (AR0.5). Removal rates of total LWCA were 80–96 % (experimental series 1), 80–93 % (experimental series 2) and 60–99 % (experimental series 3) from the initial values. After stabilization, concentrations of water-extractable LWCA ranged from 100 to 800 mg kg^{-1} dm and alkaline-extractable from 800 to 1500 mg kg^{-1} dm in all experiments. It is noteworthy, that even after 52 or 63 weeks of composting, concentrations were still at those levels.

4.2.3 Types of LWCA in composts

Out of a wide range of LWCA that were analyzed (C_1–C_6 acids in I, II, and C_1–C_{10} in III), only concentrations of formic, acetic, propionic and butyric acids were at levels above the limits of quantification. Other acids (*iso*-butyric, valeric, *iso*-valeric, caproic, enanthic, caprylic, pelargonic, and capric) could be measured qualitatively (I-III). Therefore, only the dynamics of formic, acetic, propionic and butyric acids in composting experiments are presented (Fig. 11).

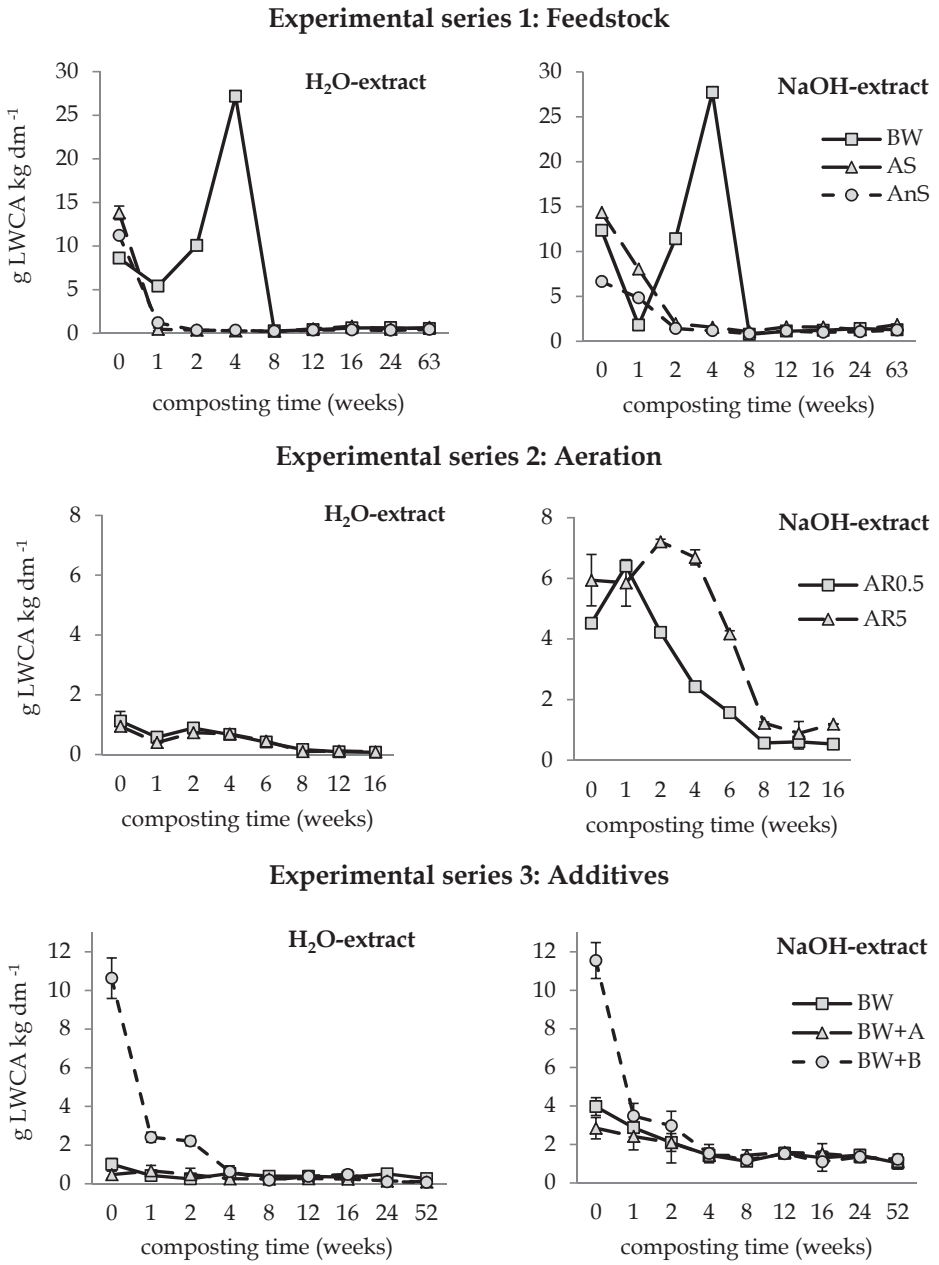

FIGURE 10 Dynamics of total LWCA extracted with water or alkaline solution from the samples obtained in the composting experiments. Composted material was: BW = kitchen biowaste/peat mixture (1/1, v/v), AS = aerobic sludge/peat mixture (1/1, v/v), AnS = anaerobic sludge/peat mixture (1/1, v/v), AR0.5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 0.5 l min^{-1}, AR5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 5 l min^{-1}, BW+A = biowaste/peat mixture (1/1, v/v) + clay-based additive A, BW+B = biowaste/peat mixture (1/1, v/v) + calcium-based additive B. Error bars indicate standard deviations of the means of four replicates.

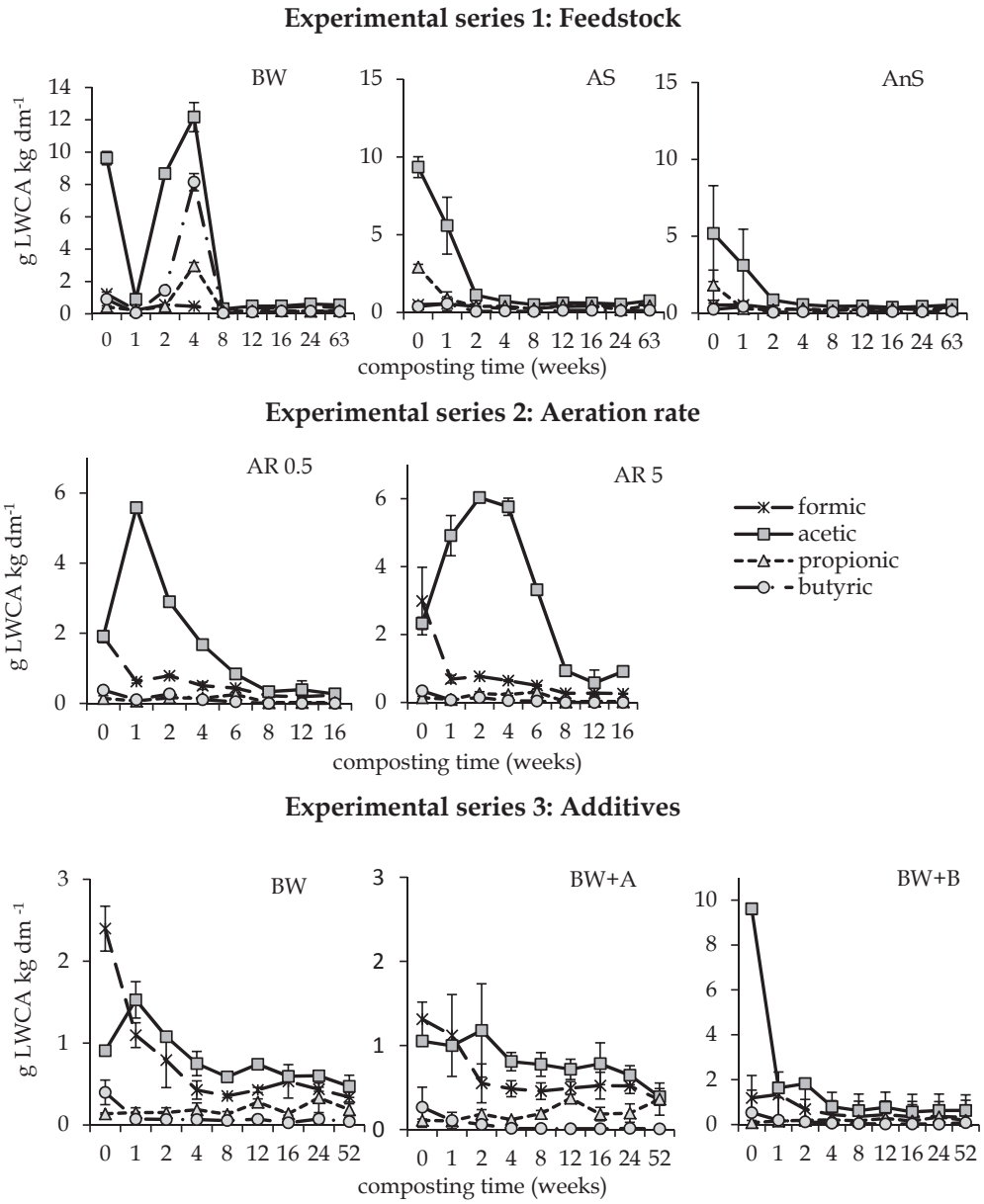

FIGURE 11 Dynamics of formic, acetic, propionic and butyric acids extracted with alkaline solution from the samples obtained in the composting experiments. Composted material was: BW = kitchen biowaste/peat mixture (1/1, v/v), AS = aerobic sludge/peat mixture (1/1, v/v), AnS = anaerobic sludge/peat mixture (1/1, v/v), AR0.5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 0.5 l min^{-1}, AR5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 5 l min^{-1}, BW+A = biowaste/peat mixture (1/1, v/v) + clay-based additive A, BW+B = biowaste/peat mixture (1/1, v/v) + calcium-based additive B. Error bars indicate standard deviations of the means of four replicates. Note different scale between the treatments in the experimental series 3.

In experimental series 1, acetic acid was dominating over the other LWCA in all three feedstocks, its concentration being 65–80 % from the total concentration of LWCA (I). In the experimental series 2 and 3, initial concentration of the formic acid was on the same level or even higher (40–60 % of the total LWCA) than the concentration of acetic acid (20–40 % of the total LWCA). Application of the calcium-based additive B led to a significant increase in the initial concentration of acetic acid (83 % of the total LWCA) (II). The amount of the propionic acid was the second highest in AS and AnS feedstock, but rather low in biowaste. In general, the concentration of butyric acid was the lowest among these four acids. As a common trend, concentrations of formic, propionic and butyric acids decreased gradually from the initial levels as composting proceeded. On the contrary, it was acetic acid that accumulated in biowaste compost during the active phase and decreased toward final values only in maturation phase.

4.3 Dynamics of phytotoxicity during composting

4.3.1 Phytotoxicity of composts evaluated by short-term assays

In short-term assays conducted on water-compost extracts out of the two measured parameters (seed germination and seedling length), germination did not indicate phytotoxicity of the compost. For this reason data on germination are not showed. In general, the number of germinated seeds was on the same level as in the control. On the contrary, early seedling growth clearly indicated phytotoxicity, which decreased with time as the composts matured.

In the experimental series 1, duration of the phytotoxic period in the sludge composts was shorter than in the biowaste compost (Fig. 12). No statistically significant differences ($p > 0.05$) between control and compost were observed after 4 weeks, 8 weeks and 12 weeks of composting in AS, AnS, and BW, respectively (I). In the experimental series 2, the phytotoxic period was significantly shorter in compost aerated at 5 l min^{-1} compared to the compost aerated at 0.5 l min^{-1}, 2 weeks and 16 weeks, respectively. In the experimental series 3, in compost BW+B the phytotoxic period was shorter compared to sole BW or BW+A, 8 weeks and 24 weeks, respectively (II).

4.3.2 Phytotoxicity of composts evaluated by subchronic assays

In subchronic assays conducted on compost-PBGM mixtures germination of the seeds was delayed to some extent, but phytotoxicity was most clearly exhibited by a delay in plant growth. For this reason data on seed germination are not shown.

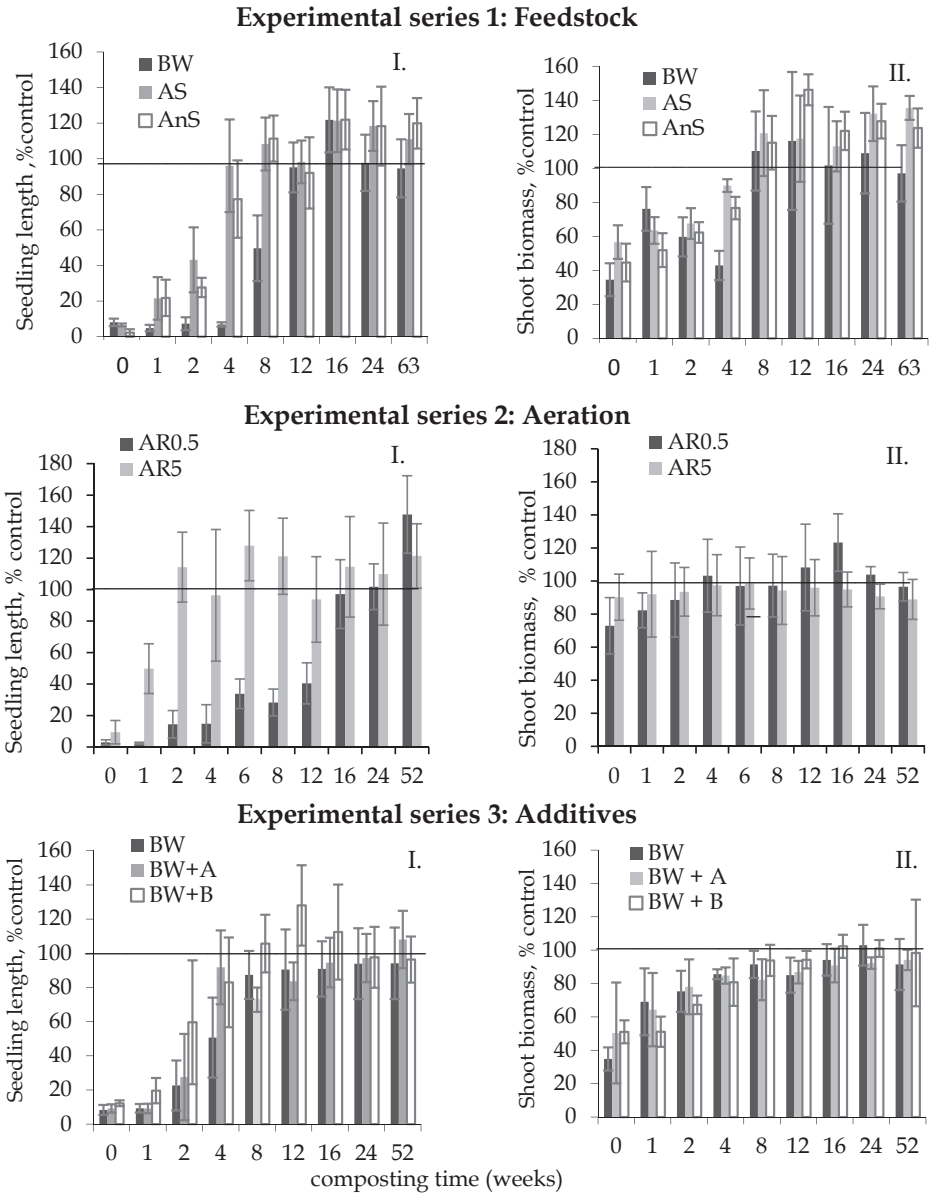

FIGURE 12 Dynamics of phytotoxicity in compost samples obtained from the composting experiments. Phytotoxicity was measured in short-term (I) and subchronic (II) assays. In I, substrates were water-compost extracts (1/2, v/v), control deionized water. In II, substrates were compost-PBGM mixtures (1/2, v/v), control PBGM. Compost was: BW = kitchen biowaste/peat mixture (1/1, v/v), AS = aerobic sludge/peat mixture (1/1, v/v), AnS = anaerobic sludge/peat mixture (1/1, v/v), AR0.5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 0.5 l min^{-1}, AR5 = kitchen biowaste/wood chips/peat mixture 6/3/1 (v/v/v) aerated at 5 l min^{-1}, BW+A = biowaste/peat mixture (1/1, v/v) + clay-based additive A, BW+B = biowaste/peat mixture (1/1, v/v) + calcium-based additive B. Error bars indicate standard deviations of the means, n = 15 (I) or n = 6 (II).

In the experimental series 1, no statistically significant differences ($p > 0.05$) in plant biomass between control and composts were observed after 8 weeks of composting in all three feedstocks (Fig. 12). In the experimental series 2, phytotoxicity was eliminated after 2 weeks in the compost aerated at 5 l min^{-1}, but took 4 weeks for the compost aerated at 0.5 l min^{-1}. However, in the former a decrease in plant growth was observed on weeks 24 and 52. In the experimental series 3, the phytotoxic period was shorter in BW+B compared to BW and BW+A, 8 weeks and 16 weeks, respectively (II).

4.3.3 Duration of phytotoxic period

By comparing the results of the two assays it can be concluded, that the duration of the phytotoxic period evaluated by short-term assays was longer than or as long as that evaluated by the subchronic assays. Depending on the case, phytotoxicity lasted from 2 to 24 weeks in the short-term assays and from 2 to 16 weeks in the subchronic assays. Both tests showed that in compost aerated at 5 l min^{-1} the phytotoxic period was significantly shorter than in compost aerated at 0.5 l min^{-1}, also the addition of the calcium-based additive B significantly shortened the phytotoxic period.

Based on the results of the composting experiments it can be concluded that, if compost is intended to be used for plant cultivation, up to 24 weeks processing might be needed in order to avoid problems with plant performance. However, a shorter or a longer time period might be needed depending on the plant species and the amount of compost applied for cultivation. It would be practical to assess the suitability of compost for plant growth in each individual case using the target species and the agricultural practice to be applied.

4.4 Effective concentration values of LWCA and mixture effect (IV, V)

4.4.1 EC values of LWCA (IV, V)

Effective concentration (EC) values were obtained in short-term and subchronic assays for LWCA with carbon chain length of C_1–C_6 (IV). As only acids with the carbon chain length of C_1–C_4 were detected in considerable amounts in compost samples, EC values for these acids are presented here. The full set of data can be found in the original publication (IV). Another set of EC values was obtained for C_1–C_3 LWCA only (V). Although dose-response assays were conducted for the same compounds, growth conditions between the two experimental sets were slightly different. Therefore, the results of each test are presented separately. Data for ryegrass shown in the publications IV and V are omitted as the species was not used in the compost phytotoxicity assays.

Among the models tested, the Weibull 1.3 (IV) or the log-logistic 1.3 (V) multiple model sufficiently described the data and was used for modeling dose–response relationships of LWCA (Fig. 13). The models were used to calculate EC10, EC50 and EC90 values for each acid for seed germination, seedling growth, and plant growth (Tables 2 and 3).

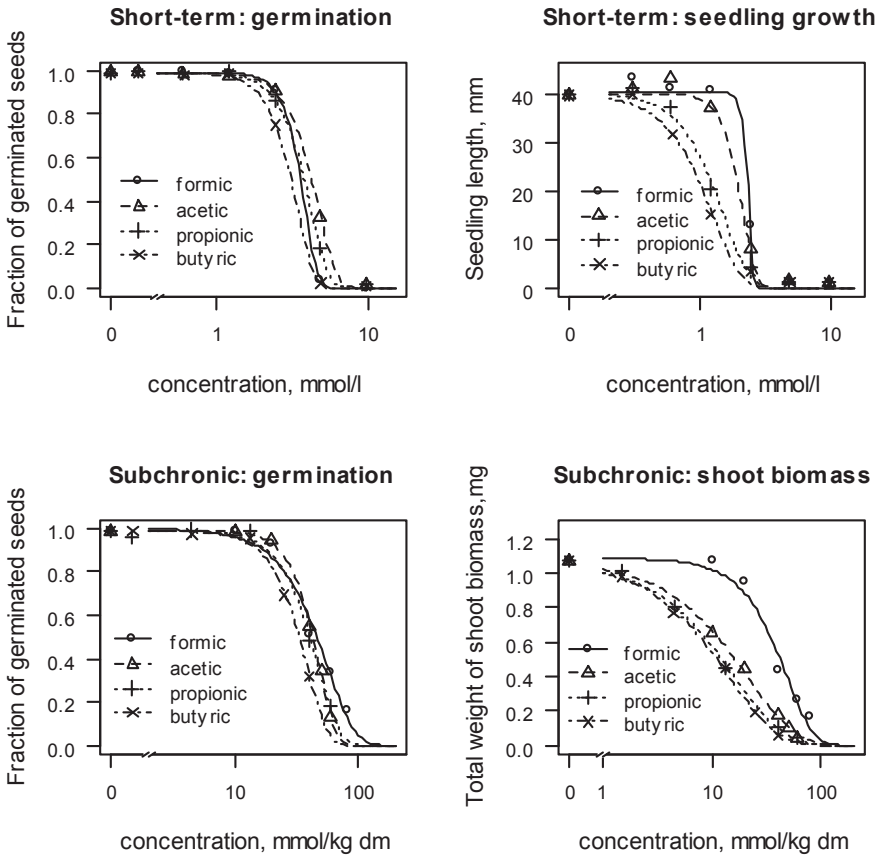

FIGURE 13 Dose-response curves of formic, acetic, propionic, and butyric acids for seed germination, seedling growth and shoot biomass of cress *Lepidium sativum* obtained in short-term and subchronic assays. The curves were acquired from the data of the experimental set I that was modeled using Weibull 1.3 model (IV).

EC50 values for C_1–C_4 LWCA obtained in short-term assays for seed germination ranged from 3.1 to 4.4 mmol l^{-1} and in subchronic assays from 25 to 46 mmol kg^{-1} dm (Tables 2 and 3). EC50 values for the early seedling growth ranged from 1.1 to 2.3 mmol l^{-1} and for shoot biomass from 10 and 40 mmol kg^{-1} dm. Both assay types showed general trend of increase in toxicity of LWCA with the increase in the carbon chain of the molecule, formic or acetic acid being the least toxic and butyric the most. The trend was also true for valeric and caproic acids (IV), being more obvious in subchronic than in acute assays.

TABLE 2 EC values of formic, acetic, propionic, and butyric acids obtained in short-term assays for reduction of seed germination and seedling growth for cress *Lepidium sativum*. The EC values were calculated using Weibull 1.3 (Exp. set 1, IV) or log-logistic 1.3 (Exp. set 2, V) model and are expressed as averages (in mmol l^{-1}), with standard errors in parenthesis. C_1–C_4 indicates number of carbons in a molecule chain of the acid.

Acid	Germination		Seedling growth	
	Exp. set 1[a]	Exp. set 2[a]	Exp. Set 1	Exp. Set 2
Formic	3.6[b] (0.1)	2.9 (0.1)	2.3 (0.4)*	1.9 (0.1)
(C_1)	2.5[c] (0.1)–4.5[d] (0.1)	2.2 (0.1)–4.0 (0.1)	2.1 (1.8)–2.5 (0.6)	1.0 (0.1)–3.7 (0.3)
Acetic	4.2 (0.1)	4.4 (0.1)	2.0 (0.1)*	2.0 (0.1)
(C_2)	2.5 (0.2)–5.8 (0.1)	3.0 (0.2)–6.4 (0.7)	1.3 (0.2)–2.6 (0.1)	0.8 (0.1)–5.2 (0.5)
Propionic	3.8 (0.1)	3.2 (0.2)	1.3 (0.1)†	1.1 (0.1)
(C_3)	2.2 (0.1)–5.2 (0.1)	1.8 (0.1)–5.6 (1.0)	0.5 (0.1)–2.2 (0.6)	0.4 (0.0)–3.0 (0.3)
Butyric	3.1 (0.1)	ND	1.1 (0.1)†	ND
(C_4)	1.9 (0.1)–4.2 (0.2)		0.4 (0.1)–1.9 (0.3)	

[a] data of the experimental sets were obtained in differed growth conditions
[b] EC50, [c] EC10 and [d] EC90
*, †– the same symbol indicates no statistically significant difference ($p > 0.05$) between EC50 values of the acids in question.
ND - not defined

TABLE 3 EC values of formic, acetic, propionic, and butyric acids obtained in subchronic assays for germination and shoot biomass of cress *Lepidium sativum*. The EC values were calculated using Weibull 1.3 (Exp. set 1, IV) or log-logistic 1.3 (Exp. set 2, V) model and are expressed as averages (in mmol kg^{-1} dm), with standard error in parenthesis. C_1–C_4 indicates number of carbons in a molecule chain of the acid.

Acid	Germination		Shoot biomass	
	Exp. set 1[a]	Exp. set 2[a]	Exp. Set 1	Exp. Set 2
Formic	46 (2.7)[b]	37 (1.2)	40 (2.1)	36 (2.9)
(C_1)	17 (2.8)[c]–86 (7.2)[d]	24 (2.8)–57 (4.5)	14 (2.0)–78 (6.1)	25 (7.0)–51 (7.4)
Acetic	43 (1.7)*	29 (1.1)	15 (1.6)	24 (1.7)
(C_2)	24 (3.1)–63 (7.2)	19 (1.2)–46 (2.4)	2 (0.7)–50 (5.7)	14 (1.9)–42 (6.3)
Propionic	41 (2.3)*	25 (NA)	11 (1.2)†	12 (1.3)
(C_3)	19 (3.6)–66 (5.5)	21 (NA)–30 (NA)	2 (0.5)–38 (6.2)	5 (1.2)–28 (6.0)
Butyric	33 (1.7)	ND	10 (1.0)†	ND
(C_4)	16 (2.5)–53 (4.9)		2 (0.4)–34 (4.2)	

[a] data of the experimental sets were obtained in differed growth conditions
[b] EC50, [c] EC10 and [d] EC90
*, †– the same symbol indicates no statistically significant difference ($p > 0.05$) between EC50 values of the acids in question.
NA – the values of the confidential interval could not be generated.
ND - not defined.

Considering EC10–EC90 intervals, transition from practically non-toxic to strongly inhibitory levels of LWCA occurred more abruptly in short-term assays than in subchronic assays. In short-term assays the interval for all end points ranged from 2 to 4 mmol l^{-1} and in subchronic assays from 23 to 70 mmol kg^{-1} dm, decreasing from formic to butyric acid. No decreasing trend in the interval magnitude was observed in the short-term assays.

The parameter EC10, if sufficiently accurately assayed, can be used as a safe level for evaluation of phytotoxicity potential of the substrate. EC10 values obtained in the subchronic assays for plant biomass may be used as suitable end point in the evaluation. From the toxicological point of view concentration expressed as molality (mmol kg^{-1}) is more informative for comparisons, although, concentration expressed as mass fraction (mg kg^{-1}) is more commonly used. Therefore, for easier utilization of the toxicological data, the EC values are expressed in both forms. Thus, taken as average values from the two experimental sets (IV and V) EC10 values were (average ± st.dev.): formic acid = 20 ± 5 mmol kg^{-1} dm (920 ± 230 mg kg^{-1} dm), acetic acid = 8 ± 6 mmol kg^{-1} dm (480 ± 360 mg kg^{-1} dm), propionic acid = 3.5 ± 1.5 mmol kg^{-1} dm (270 ± 117 mg kg^{-1} dm) and butyric acid = 2 ± 0.4 mmol kg^{-1} dm (410 ± 35 mg kg^{-1} dm).

4.4.2 Type of toxicity in LWCA mixtures (V)

In binary mixtures, for all assay endpoints (germination, early seedling growth and biomass production) EC50 values were around 1 TU, the means ranging from 0.8 to 1.4 TU for F+A mixture and from 0.6 to 1.2 TU for F+P and A+P mixtures (V). In ternary mixtures, EC50 in short term assays was slightly lower (range between 0.4 and 1.0 TU) than in subchronic assays (range between 0.9 and 1.3 TU).

For most endpoints, the interaction index of binary mixtures analysis showed an additive value of around one. The range of the index limit values was slightly negative or overlapping zero, meaning no unexpected type of interactions. Thus, toxic unit and interaction index analysis suggested a dose addition mechanism in LWCA mixtures, which means that each acid act separately and no interactions occur due the acid characteristics or other reason. Therefore, phytotoxicity of the substrate depends on what LWCA are present in the growth substrate and what is the concentration of each acid.

4.5 Evaluation of compost phytotoxicity due to LWCA (I-V)

4.5.1 Non-phytotoxic concentrations of LWCA in compost

For evaluation of the practically non-phytotoxic level of LWCA in compost, it is customary to use EC10 values that indicate a 10 % decrease in plant production compared to the control. According to the national Decree 1784/14/2011 on fertilizers issued by the Finnish Ministry of Forestry and Agriculture, a 20 %

decrease in germination index of cress is acceptable for compost used as soil improver. Taking into account the additive nature of phytotoxicity of LWCA in mixtures and variability in the results (IV, V), EC10 value for the molar equitoxic LWCA mixture would be 8.4 ± 4.2 mmol kg^{-1} dm (520 ± 190 mg kg^{-1} dm). Generalizing, it can be suggested that a nontoxic concentration of LWCA is somewhat less than 12.6 mmol kg^{-1} dm (800 mg kg^{-1} dm).

4.5.2 Correlation of LWCA concentrations and the phytotoxicity of composts

If we accept 800 mg kg^{-1} dm as a nonphytotoxic level for LWCA, at concentrations measured in compost below that level shoot biomass index in subchronic assay corresponded to 62–142 % and for concentrations above it, the index was 34–103 % (Fig. 14). In the samples, where concentrations of LWCA were below 800 mg kg^{-1} dm there were only two cases where plant growth was significantly lower than in control (62 and 77 %) and both samples belonged to anaerobic compost after two and four weeks of composting. However, there were quite many occasions, when plants were growing well at concentrations of LWCA above 800 mg kg^{-1} dm and even as high as 4.0 g kg^{-1} dm.

Results of the assays showed that LWCA can exhibit phytotoxicity. Although concentrations of LWCA in compost were measured at grams kg^{-1} dm level, phytotoxicity of composts due to LWCA can be explained only to some extent. Spearman's rank correlation coefficients of the results obtained in phytotoxicity assays and concentrations of LWCA measured in composts ranged from –0.77 to –0.51 (Table 4). The correlation coefficient was highest for LWCA extracted with alkaline solution and phytotoxicity measured in subchronic assays (–0.77). Correlation coefficients between individual acids and phytotoxicity were also rather low. The coefficients from the experimental series 1 and 3 are presented in the original publications (Table 5 in I and II) and coefficients from the experiment 2 ranged from 0.1 to 0.6.

TABLE 4 Spearman's rank correlation coefficients (ρ) of LWCA concentrations and results of the phytotoxicity assays conducted on compost samples obtained in the composting experiments.

	Short-term assay	Subchronic assay
LWCA extracted with water	–0.53	–0.51
LWCA extracted with alkaline solution	–0.72	–0.77

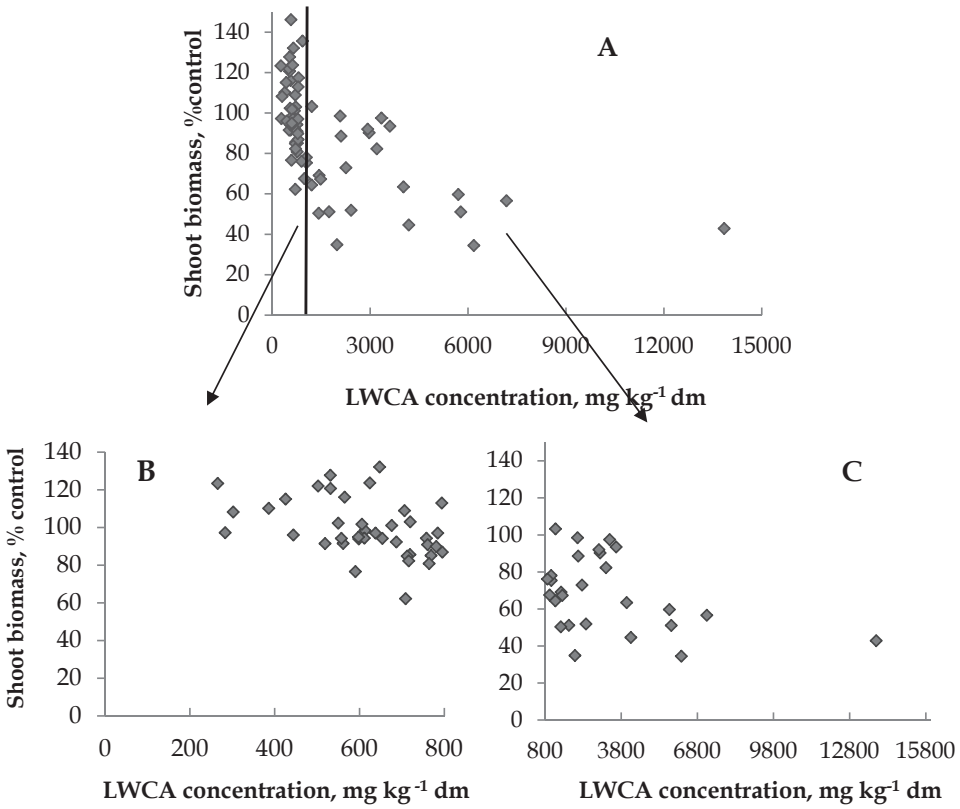

FIGURE 14 A = Correlation of LWCA concentrations extracted with alkaline solution from compost samples obtained in the composting experiments and plant biomass obtained in subchronic assays (A). B = the correlation for LWCA concentrations < 800 mg kg^{-1} dm and C = the correlation for LWCA concentrations > 800 mg kg^{-1} dm (C).

5 DISCUSSION

5.1 The composting process – role of feedstock, aeration and additives

5.1.1 Instability of the feedstock – the reason for the process activity

Instability of organic matter in the feedstock in terms of its biological degradation potential is an important aspect of the process. The less feedstock has been processed before composting, the more actively it is degraded during the subsequent process. Before composting, organic matter in the aerobic and anaerobic sludges has gone through degradation processes during the activated sludge treatment of the wastewater (in AS) and, additionally, in anaerobic treatment of the activated sludge (in AnS). Thus, during each treatment, organic matter becomes exhausted to some extent as less easily biodegradable compounds are available to microorganisms for the next stage. Among the feedstocks tested (biowaste, municipal aerobic and anaerobic sludges), BW had the highest activity compared to the sludges, expressed by the higher temperatures, higher CO_2-release, ammonification and nitrification, and more intensive dynamics of LWCA (I). Therefore, the degree of organic matter exhaustion in the feedstock defined how actively the process progressed. Comparative studies on the role of substrate exhaustion on composting in the scientific literature are lacking as, usually, either biowaste or sewage sludges are used as feedstocks in experimental setups. However, the experience of the composting plant operators in Finland showed that this aspect of the substrate exhaustion was not taken into account when composting of the source-separated biowaste was implemented. Composting of biowaste in Finland started in the early 1990's, while composting of wastewater sludges has been carried out since 1970's in open windrow systems. In the early days composting of at-source separated biowaste was also carried out in windrows and met with problems due to foul odours and residents' complaints in the surrounding areas. With hindsight, if timely more research had been conducted on this

aspect, large economic losses and social tensions could have been avoided, and cost-effective technological solutions could have been found.

5.1.2 Aeration: The more the better?

Being the crucial parameter of composting, oxygen availability and aeration has gained much attention in research (Barrington *et al.* 2003, Sundberg and Jönsson 2008, Guo *et al.* 2012). Many researchers, as well as compost producers, support the idea that aeration should be at the highest possible level to provide proper composting (de Bertoldi *et al.* 2000), however recommendations on optimal concentrations of oxygen during composting vary significantly. Nevertheless, not maximization but optimization of the aeration should be the aim for composting as, in addition to oxygen supply, aeration is an important tool in controlling moisture and heat. Therefore, balancing these three parameters needs a lot of experience and understanding of the process. According to the results of experimental series 2, an aeration rate of 5 l min^{-1} (AR5) can be considered as optimal, because at this rate organic matter was mineralized more rapidly, pH was stabilized more quickly and phytotoxicity was removed earlier than in compost with a low aeration rate (AR0.5). Excess drying of the mass was avoided as moisture content fluctuated between 25 % and 30 %, which was on the lower edge of the optimal values (Keener *et al.* 2000) but did not seem to disturb the process. On the other hand, LWCA were accumulated in higher amounts and were eliminated more slowly in compost AR5 than in AR0.5. A drawback of the experimental design was that oxygen supply was monitored only by the total aeration rate and sequestration of oxygen concentration at micro levels was not measured. In future studies this should provide a more valuable set of information on the processes inside the degrading mass.

For recycling of organic waste and compost operators, mineralization of OM is the primary aim, however according to Binner et al. (2010) fast mineralization is not favourable for humification processes. Therefore, if compost is produced with the purpose of utilization as a soil improver, aeration should be optimized taking into consideration this aspect, too.

5.1.3 Role of additives in composting

Application of additive with a high content of calcium hydroxide and the presence of calcium peroxide did not have an effect on the maximum temperatures, but caused quicker stabilization of pH and additionally led to faster elimination of water-soluble NH_4-N and an increase in NO_3-N, thereby enhancing stripping of ammonia (II). A similar effect on pH and NH_4-N dynamics was observed by the addition of lime or fly ash (Fang, 1999).

In the compost with clay based additive, 5–10 °C higher temperature between second and third weeks was measured, however, no other difference compared to the control was observed. Similar results with this additive were observed by Korhonen (2006).

In conclusion, based on results of this study, calcium-based additive had a significant impact on the measured parameters, while the effect of the clay-based additive was not obvious. An improvement on this study could be the measurement of humic substances that are claimed to be an important indicator for the effectiveness of the applications of clay-based additive.

5.2 LWCA dynamics during composting

5.2.1 Analytical aspects of LWCA quantification

Extraction of LWCA with alkaline solution was more effective than with water as concentrations measured in water extracts were, on average, 10–50 % from those measured in alkaline extracts. However, besides the acids dissolved in the water phase or adsorbed to the compost matrix, extraction with alkaline solution may break down ester bonds of LWCA and alcohols and, thus, cause overestimation of the LWCA amounts. This might be the case especially for immature compost, where the esters of LWCA are abundant (Mikkola *et al.* 2003).

Although derivatization of LWCA with further GC-FID detection is an accurate and effective method, it turned out to be very laborious and time-consuming. Modern analytical techniques using, for example, mass spectrometer (MS) detection offers major advantages for specific applications due to less laborious sample pre-treatment and their potential to combine high sensitivity with mass selectivity. A group of quick methods (HPLC-APCI-MS and IC-CD) that were developed recently for detection of carboxylic acids in black liquor (Käkölä and Alén 2006, Käkölä *et al.* 2008) may also have application to LWCA analysis in compost.

5.2.2 Does presence of LWCA indicate unsuccessful composting?

The presence of LWCA in compost does not necessarily indicate unsuccessfully managed process. It reflects more the process activity, rather than poor process design as claimed by many researchers (de Bertoldi *et al.* 2000, Partanen *et al.* 2010). Alterations of aerobic and hypoxic biotransformations resulting in accumulation of the LWCA are unavoidable in big volumes of easily degradable solid substrates. Definitely, the availability of O_2 at the micro-levels provides quick removal of the LWCA from the process. According to the results of this study, the defining factor for LWCA build-up was instability of the feedstock. Accumulation of LWCA was observed in all biowaste composts but not in the sludges, where concentrations merely decreased from the initial levels. Stronger aeration did not prevent LWCA accumulation, although the opposite had been expected. Higher concentrations of LWCA for a longer period of time were recorded in the compost aerated at a high rate compared to the compost aerated at a low rate, although moisture conditions in the former

were better optimized than in the latter. Accordingly, no significant impact of the aeration on LWCA concentrations was observed by DeVleeschauwer *et al.* (1982) during composting of town refuse and by Wang *et al.* (2002) during treatment of kitchen garbage. On the contrary, Robertson (2002) showed almost immediate removal of LWCA when the aeration was applied during 30-days composting of a vegetable-sawdust mixture. However, the study was conducted in mini-scale reactors (vol. 3 l) and it may be supposed that in this scale the stripping of LWCA was more efficient than in larger volumes of composting mass.

The application of calcium-based additive caused an immediate 10-fold increase in the concentration of the acetic acid with no effect on other acids. This can be explained by O_2 release resulting from chemical transformation of the active compound of the additive, calcium peroxide, supporting incomplete oxidation rather than complete oxidation of the substrate. The effect of the compound as an additional source of O_2 was quite short as the concentration of acetic acid dropped significantly during the first week with further dynamics being similar to the control, where small amounts of acetic acid were built up between weeks one and two. Quick removal of acetic acid could be also explained with high microbial activity, defined by neutral, more optimal, pH achieved by application of the additive. No fast removal of LWCA, as in this study, due to calcium-based additive application, was registered after 60 days of sewage sludge composting (Mikkola *et al.* 2003).

The unpleasant odour of LWCA is a subjective aspect, so in the perception of humans the process is unsuccessful if LWCA are formed. However, for the bacterial community LWCA is a valuable resource both for aerobic and fermentative microorganisms. Thus, the aim of the composting technology should be prevention of LWCA escaping from the system, in order to decrease the negative impact on human communities, and support their degradation in the system. In this case, excessively strong aeration increases the stripping of LWCA from the system adding problematic malodours, while optimal aeration supports their removal in a natural way. To keep LWCA in the system, physical properties play an important role. For example, keeping the pH of the mass at slightly basic values can decrease stripping of LWCA from the compost (Wiles *et al.* 2000). Carrying out of composting in a closed system is also one way to avoid foul odour problems in the surroundings of the composting facility.

5.2.3 Types of LWCA in composts

Although LWCA belong to the same group of monocarboxylic acids, the dynamics of individual acids during composting are different. Results of the study provided data on the dynamics of formic acid during composting in addition to the other LWCA. In some cases the concentration of formic acids in the feedstock was as high as acetic (II), meaning that formic acid entered the process with the feedstock, both sludges and biowaste. However, its dynamics were similar to propionic and butyric acids, i.e. concentrations gradually decreased as composting proceeded. On the contrary, it was acetic acid that

accumulated during the active stage of biowaste composting indicating the active degradation of the substrate. The result was expected as the build-up of acetic acid during composting has been presented earlier (Lynch 1977, DeVleeschauwer *et al.* 1982, Wiles *et al.* 2000). The acetic acid is the product of incomplete oxidation and fermentation of many substrates and in many biochemical pathways while formic, propionic and butyric acids are more specific for particular substrate or degradation pathways. Therefore, the presence of the acetic acid may serve as an indicator of the process activity but not necessarily availability of the O_2 in the substrate.

5.2.4 Formation of the LWCA pool in mature compost

The formation of LWCA during composting can be considered transient and the compounds should be completely removed in the active phase. However, the results of the current experiments showed that concentrations of LWCA decreased significantly as the process proceeded, but did not fall below detectable limits and eventually formed a pool in the mature compost. Concentrations of LWCA stabilized and fluctuated at 100–800 mg kg^{-1} dm (for water-extractable) and 800–1500 mg kg^{-1} dm (for alkaline-extractable). The pool was formed and remained in mature composts regardless of the feedstock type, aeration rate or additives applied. Similar results were obtained for town refuse compost, where 200 ppm of acetic acids was measured in the 20 weeks old compost, being, however, only a trace from the initial 19 000 ppm (DeVleeschauwer *et al.* 1982). During composting of olive tree leaves for 25 weeks, concentrations of organic acids in water-compost extract decreased from 528 to 112 ppm (Manois *et al.* 1987). Accordingly, the decrease in LWCA concentrations by 87 % was reported after 21-day composting of manure-sawdust compost (Wiles *et al.* 2001).

Pool formation of LWCA in mature compost can be explained by: 1) bonding to organic matter during humification process and becoming unavailable as substrate for microbial degradation, but remaining analytically detectable and 2) excretion by fungi as result of decomposition of the less readily degradable substrates. Binding of LWCA to humic substances may happen already during active phase of composting when compounds are formed in excess and are not degraded by microorganisms. Although humic substances are characterised by dominant negatively-charged sites, they are also rich in hydroxyl- and amide-groups that may bind LWCA through esterification and ammonification reactions. Additionally, humic acids of composts form complexes with metals such as Zn, Cu, Fe, Al, Pb (Miikki *et al.* 1997, Ruiz-Haas *et al.* 1998) that may also serve as acceptors for carboxylic radicals. Fungi, in turn, are the dominant microorganisms during maturation of compost and their metabolism is strictly aerobic, but they may conduct incomplete oxidation if carbohydrates are available in excess. However, the dominant metabolic product is lactic acid with smaller amounts of formic, acetic and other organic acids. Additionally, in natural habitats, e.g. soils, no

significant excretion of intermediate products to extracellular space was recorded (Schlegel 1986).

5.3 Phytotoxicity of composts

5.3.1 Evaluation of toxicity with short-term and subchronic assays

The results of the short-term and subchronic assays cannot be compared directly. The short-term assay in this study was developed in analogue to the widely used germination index method presented by Zucconi *et al.* (1981) and was conducted on water extract of composts with duration of 48 hours. Its main advantages are simplicity and quickness, and, due to its popularity, an abundance of reference data. However, results obtained on the water extracts are not reliable enough for estimation of the full phytotoxicity potential of the solid substrate, because the extraction process adds its uncertainty to the evaluation process. Instead, subchronic assays conducted on the solid substrate, provide more reliable data for phytotoxicity evaluation, but it takes 21 days before the results are ready. As a rule, phytotoxicity measured by subchronic assays was eliminated after a shorter composting period compared to the phytotoxicity evaluated by short-term assays. The opposite results were obtained by Levy and Taylor (2003) who observed toxicity of municipal solid waste compost in 63 days plant growth assays on tomatoes and no phytotoxicity of compost extracts in 5 days assay on radish. To overcome the uncertainty due to extraction, a short-term bioassay on whole-compost substrate may be a suitable solution. Results of such an assay can be obtained rather quickly as the assay only lasts for several days. However, short duration of the test will not eliminate uncertainty for estimation of the substrate phytotoxicity in a long run. Observations by Oleszczuk (2008) on raw and composted sewage sludge showed that phytotoxicity was higher in short-term 3 days assay compared to subchronic 14 days assay.

Between the two end points measured in each bioassay, seed germination was a less sensitive parameter than plant growth (seedling length or plant biomass) as no significant difference was observed between controls and treatments. Similar results were obtained on yard trimming compost assayed with barley and zucchini (Brewer and Sullivan 2003), 15 contaminated soils tested with four species (oat, cress, turnip, and bush bean) (Gong *et al.* 2001) and on raw and composted sewage sludge assayed with cress (Oleszczuk 2008). However, phytotoxicity of compost extracts for both germination and seedling growth has been reported in many studies using different species like Chinese cabbage (Wong and Chu 1985, Tiquia and Tam 1998), Chinese spinach (Tiquia and Tam 1998), cress (Tiquia 2010), carrot (Wong and Chu 1985) and tomatoes (Levy and Taylor 2003). Brinton and Evans (2001) observed decrease of germination for cress and no effect for wheat. The difference between results of the studies may be explained by: 1) variability in species' demand for

environmental conditions during the germination process and 2) the way germination was measured. Many factors, like water and oxygen availability, temperature, light intensity and light-darkness regime and, stimulating or retarding compounds in the substrate affect the process of seed germination. Therefore, seeds of one species may be more sensitive to environmental conditions during germination than another and indicate phytotoxicity in a different way. In addition, method by which the seed is recorded as germinated is an important aspect. In plant growth assays germination is recorded when the cotyledon is visible above the substrate. In the assays conducted on extracts, germination is more subjective. For example, in this research cress seed was accepted as germinated when the length of the primary root > 1 mm, while in the research of Tiquia (2010) it was > 5 mm. So, if a compost extract were to contain compounds that do not suppress seed germination but only retards primary root growth, difference in germination measurement method may have an impact on the final result. Comparison of the results between the studies is often very difficult as criteria for germination success are not always reported by the authors. Therefore overall, standardized procedures for measuring phytotoxicity are very important both in scientific studies as well as for composting plant operators for assessment of the compost quality.

5.3.2 Dynamics of phytotoxicity during composting

The dynamics of the phytotoxicity in the three composting experimental series was typical for composting. In the beginning the compost was phytotoxic, and, while degradation progressed, the phytotoxicity was eliminated and compost gained features that supported plant growth. Depending on the phytotoxic bioassay and the experimental setup, the phytotoxic period lasted from two to 24 weeks. The period of toxicity was similar to the findings obtained by Zucconi *et al.* (1981), Wong and Chu (1985), and Tiquia and Tam (1998). In the research of Hartz and Giannini (1998), at least 9 to 12 weeks were required to minimize the negative impact of immature municipal yard compost. At least 17 weeks of composting were needed for town refuse before it could be safely utilized as a plant growth substrate (DeVleeschauwer *et al.* 1982). From 16 to 20 weeks of processing were needed to obtain nonphytotoxic compost from olive-mill by-product "alperujo" (Alburquerque *et al.* 2006).

The results of both assays showed that higher aeration rate and application of calcium-based additive significantly reduced the phytotoxic period. Shortening of the phytotoxic period due to increased aeration was reported earlier (Zucconi *et al.* 1981b). However, no significant impact of the aeration rate on the germination index was found by Guo *et al.* (2012) during composting of pig faeces and corn, or by DeVleeschauwer *et al.* (1982) during composting of town refuse. Possibly, in those studies aeration was high enough to strip LWCA out of the mass, thus no correlation between aeration and phytotoxicity was observed. Comparative data on the effect of calcium-based additive is lacking.

5.3.3 Phytotoxic levels of LWCA in compost

Phytotoxicity assays affirmed that LWCA are phytotoxic and, therefore, may be the reason for compost phytotoxicity. Then comes the question on what are the phytotoxic concentrations of LWCA in compost as already in the 15th century Paracelsus stated: "*All substances are poisons; there is non that is not a poison. The right dose differentiates a poison and remedy*" (Timbrell 2000). Thus, three main questions may arise:

- How phytotoxicity potential changes, when several acids are present?
- Are the concentrations of LWCA as mixtures high enough to cause phytotoxicity in immature compost?
- Are the concentrations of the LWCA as mixtures high enough to cause phytotoxicity in mature compost?

Results of the experiments on mixture phytotoxicity of LWCA suggested dose addition mechanism, which means that each acid act separately and no interactions occur due to the acid characteristics or other reason. Therefore, phytotoxicity of the substrate depends on what LWCA are present in the growth substrate and in what concentration. According to obtained results, role of the carbon chain length becomes more important in mixture toxicity, i.e. mixture of acids with longer chain is more phytotoxic than mixture of acids with shorter chain. For example, in short-term assay EC50 value of sole acetic acid was 2.0 mmol l^{-1} (95 % conf.int 1.8–2.2), which is higher than 1.0 mmol l^{-1} (1.0–1.1) in A+F, 0.8 mmol l^{-1} (0.7–0.8) in A+P and 0.6 mmol l^{-1} (0.5–0.6) in F+A+P. However, this trend was not so obvious in subchronic endpoints. Increase of toxicity in mixture compared to the pure acids was observed for acetic, propionic and butyric acids (Schuman and McCalla 1976). In the study germination of wheat in pure acids was 73–89% and in the equimolar mixture 51% and of sorghum 53–67 % and 49 %respectively. The trend may suggest that in a mixture each LWCA has its own relative potency (REP) value that increases with the increase of the carbon chain. Similar suggestion was made by Lynch (1977) stating that at equivalent concentrations, the phytotoxicity of propionic and butyric acids was greater than that of acetic acid by factors of about 2 and 3, respectively. If this hypothesis is true propionic and butyric acids still make a contribution to the phytotoxicity of compost, although they present in small concentrations. However, more research is needed in order to appoint PER values for LWCA and more data on phytotoxicity potential of C_4–C_6 should be collected.

Based on the results of the dose-response studies on pure LWCA and additive effect in mixture, it can be concluded that concentrations of LWCA in immature compost were high enough to cause phytotoxicity. As a rule, the concentrations of LWCA exceeded the EC10 values that may be considered as practically acceptable safe levels. Thus, for the equimolar mixtures, the EC10 would be around 800 mg^{-1} kg dm. It is worthy of note that the value should be accepted as approximate, because it was obtained based on the nominal concentrations of LWCA that were added to the initial growth substrate but not

on the concentrations analyzed at the end of the assay. The actual effective concentrations may be lower as phytotoxicity was registered in spite of possible LWCA volatilization or degradation during the assays. Another aspect is the proportion of different acids present on different stages of composting. It could be seen that acetic acid was usually dominating over the others in immature composts, meaning that at this stage compost phytotoxicity was mainly defined by acetic acid. Instead, in mature compost concentrations of all acid types were nearly at the same level, so the equimolar toxicity concentrations could be applied in this case. Information on the phytotoxic levels of LWCA in composts is very scarce. A total concentration of 1250 mg kg in compost-containing growth media was suggested as non-toxic by Brinton and Tränkner (1999), which is slightly higher than obtained in this research.

It should be understood that in environmental practice a wider range of concentrations rather than a narrow one may be accepted as LWCA nonphytotoxic levels due to high instability and biodegradability of the compounds on site. There were experimental situations when no phytotoxic effect was observed at the concentrations as high as 4000 mg^{-1} kg dm. Possibly, although high concentrations of LWCA were detectable, the acids were bound to organic matter and were not readily available for plants. Thus, although a pool of LWCA as high as 800–1500 mg kg^{-1} dm (for alkaline-extractable) was measured in mature compost, it falls within a range of safe concentrations, although is slight higher than the value suggested in the study.

5.3.4 Effects of LWCA on plants

LWCA are natural compounds that are widely synthesized and metabolized in plants. It was demonstrated, that acetate-C^{14} adsorbed by plant roots was partly mineralized into CO_2, partly incorporated into tricarboxylic acid cycle, converted into soluble cell constituents and insoluble material like cell wall and proteins (Harley and Beevers 1963, Tsuda 2012). Acetyl-CoA plays important role in many biosynthesis and biodegradation pathways (Buchanan *et al.* 2000). Formate in plants serves as main precursor for biosynthetic reactions via the mevalonate pathway and additionally its metabolism is closely related to serine synthesis (Igamberdiev *et al.* 1999).

However, when concentrations of the LWCA in growth substrate are high enough, they interfere with plant growth that was demonstrated by phytotoxic assays. Mechanisms of LWCA phytotoxicity is not clearly understood, yet. There is no common agreement whether dissociated or undissociated form of LWCA is phytotoxic. The toxicity of LWCA can be regarded as partly due to H^+-ion and partly due to undissociated acid or dissociated anion (Stiles and Rees 1935). According to the uncoupling theory, at low pH toxicity is defined by the undissociated form (RCOOH), which is membrane-permeable, therefore, toxicity of more lipophilic compounds is higher than less lipophilic as they can easier enter the root cells (Russell and Diez-Gonzalez 1998). Once the molecules enter the intracellular space, they partly dissociate or stay in molecular form as pH of near-cell wall solution is 5 (Tiaz and Zeiger 1998), however complete

dissociation occurs in cytosol, where pH is about 7.5 (Buchanan *et al.* 2000). This leads to pH drop and further affects e.g. enzymatic activity. If suppose that toxicity is mainly defined by the undissociated forms, no toxic effect should be observed if pH of growth substrate is close to neutral, when LWCA are fully dissociated. However, Chandrasekaran and Yoshida (1973) found adverse effects of LWCA on rice growth at neutral pH (7.1–7.5) at concentration 5 mmol kg^{-1} soil. Penetration of dissociated form of formic and acetic acids through the membrane at pH = 6.4 was demonstrated by Jackson *et al.* (1970). Dose-response effect of C_1–C_7 LWCA at neutral pH on wheat *Triticum* sp was demonstrated by Prill *et al.* (1949). No appreciable difference between inhibition of either radicle emergence or root growth was observed at pH 4.8 and at pH 6.0 for C_5–C_7 acids (Ulbright *et al.* 1982). Thus, dissociated form of LWCA may exhibit phytotoxic effect also.

One suggestion for LWCA toxicity mechanism is disturbance of osmotic regulations of root cells. Once the molecules have entered the root cells, they change permeability of the membrane and cause hyperpolarization of transmembrane electrical potential. This leads to rapid loss of K^+, Ca^{2+}, and Cl^- ions into the external substrate and flow of Na^+ ions into the cell that was demonstrated in many experimental studies (Jackson and Taylor 1970, Lee 1977, Marrè *et al.* 1983). Interference with membrane permeability, in turn, causes leakage of protons across the cell membrane which leads to acidification of the cytoplasm and inhibition of nutrient transport (Cherrington *et al.* 1991). Another possible toxic mechanism is that LWCA mimic plant hormones auxins that have carboxyl radical as an active constituent in their structure (Tiaz and Zeiger 1998). The hormones promote cell growth in stems and coleoptiles but inhibit in roots by inducing synthesis of growth inhibitor ethylene. If LWCA are transported into stems, they may also mimic antiauxins by blocking a carboxylic acid-binding site of auxin-binding protein 1 (ABP1), thus disturbing growth of stem cells (Tiaz and Zeiger 1998). Another possibility is that LWCA induce the oxygen loss from the apical regions of the roots and, thus, increase cell wall lignification that leads to early maturation of roots (Armstrong and Armstrong 2001).

5.3.5 LWCA - the major reason for toxicity of immature compost?

The correlation between compost phytotoxicity and concentrations of LWCA have been reported earlier in many studies, suggesting that LWCA is the main cause of compost phytotoxicity (Chandrasekaran and Yoshida 1973, Chanyasak *et al.* 1982, DeVleeschauwer *et al.* 1982, Brinton and Tränker 1999). In this study correlation of compost phytotoxicity and LWCA concentrations (extracted with alkaline) was –0.77 suggesting that phytotoxicity due to LWCA can be explained to some extent only. The reason for moderate correlation could be that 1) LWCA of compost in assays were not available to plants to cause strong phytotoxic effect or 2) there were other reasons for compost phytotoxicity than LWCA.

In the plant assays compost was extracted with water or mixed with PBGM, which might have caused sorption of LWCA to dissolved organic matter (in extract) or to peat (in solid substrate). It is know that both matrices have high absorbance capacity and may partly reduce LWCA availability to plants. If the assays had been conducted on undiluted compost samples, the matrix effect could have been avoided and dissimilar results could have been obtained. Phytotoxicity of composts due to other compounds, such as ammonia, phenol compounds, ethylene oxide or heavy metals have also been discussed in the literature. In the present study, the possibility of phytotoxicity due to ammonia is low as correlation coefficients for ammonia were lower than for LWCA (I, II). Concentrations of heavy metals in composts were also low (I, II). Concentrations of neither phenolics nor ethylene oxide were measured in this research, therefore their role in compost phytotoxicity cannot be evaluated. Aslam and VanderGheynst (2009) observed strong correlation between phytotoxicity and remaining degradable carbon. Their suggestion may explain results obtained especially in the experimental series 2, where compost in AR5 was not phytotoxic although concentrations of LWCA were high (weeks 2–6). Thus, stronger aeration caused higher degradation of organic matter and lower content of the remaining carbon leading to quicker removal of phytotoxicity. Therefore, the possibly of the presence of organic compounds other than LWCA could be an additional reason for phytotoxicity of immature composts, needing more research to prove it.

5.4 Further studies

This thesis expands knowledge of the dynamics of LWCA during composting processes and on role of LWCA in phytotoxicity of composts. However, some additional research may be needed for a more comprehensive understanding of the compost phytotoxicity phenomenon as part of the composting process in general. Such issues should be addressed as:

- Behaviour of LWCA during composting based on physical and chemical properties. This aspect should gain more attention in order to understand better volatilization of the compounds from compost, the mechanism of their binding to OM and other possible transformations.
- Phytotoxicity of metabolic acids other than LWCA, e.g. from di-carboxilic and hydroxylic groups. The dynamics of these compounds should be studied as they are formed and excreted during microbial degradation of OM. Results of the phytotoxicity assays would give a better picture of the phytotoxic levels if concentrations of compounds are analyzed at the beginning and at the end of the assays in the substrate. Use of modern analytical methods allows fast analysis of large sample series.

- Effect of aeration rate on delivery of oxygen to micro-sites during composting and formation of anoxic conditions due to channelling of the substrate in case of strong aeration.

6 CONCLUSIONS

The aim of this thesis was to study phytotoxicity of compost at different stages of the composting process and to evaluate the role of the LWCA in toxicity. As the first part of the research, the effects of parameters like feedstock, aeration and additives on phytotoxicity and LWCA dynamics were studied. Another aspect was determination of the phytotoxic levels of pure LWCA and evaluation of their mixture toxicity.

The results of composting experiments showed that the degree of organic matter instability of the feedstock defined the intensiveness of the composting in the active phase. Composting of at-source separated biowaste proceeded more actively compared to the municipal wastewater sludges. A higher degree of OM instability also defined more active dynamics of LWCA. During the active phase of composting LWCA accumulated in biowaste compost, while in the sludges concentrations gradually decreased from the initial values. The experiments also showed that stronger aeration did not prevent accumulation of the LWCA in compost, contrary to widely accepted opinion. Higher amounts of LWCA were built up in compost aerated at a high rate than at a low rate. This proves the importance of oxygen availability on spatial levels rather than total amount of air or oxygen passing through the system. In addition, accumulation of LWCA was difficult to prevent in unstable feedstock as LWCA formation was not solely the result of anaerobic degradation, but an outcome of incomplete oxidation and fermentative respiration. Application of calcium-based additive led to an immediate boost in formation of acetic acid with no significant impact on other LWCA; additionally it provided earlier stabilization of pH. No significant effects on the measured parameters were observed after application of clay-based additive.

Analytical procedures developed in this study allowed precise measurements of LWCA and the obtention of valuable data on the presence of formic acid in compost. Accurate analysis allowed a better picture of the dynamics of the LWCA in immature compost to be obtained and, what is more important, in mature compost as such data is lacking in the earlier studies. Results supported the findings that acetic acid is the most abundant

intermediate product and it tends to accumulate during the active phase of composting. In turn, the dynamics of formic acid were similar to the dynamics of propionic and butyric acids, whose concentrations decreased gradually while composting proceeded.

The dynamics of phytotoxicity which were followed during the composting experiments showed that immature compost was highly phytotoxic and turned into a valuable plant growth substrate as the process progressed. The phytotoxic period lasted from two to 24 weeks depending on the process design. Stronger aeration and application of the calcium-based additive significantly shortened the phytotoxic period. Dose-response models from experiments on pure LWCA and their mixtures allowed definition of an EC10 value that can be considered as a practically safe level for plants, revealing a value of 800 mg kg^{-1} dry matter. Total concentrations of LWCA in immature composts were high above the EC10 values suggesting that LWCA were the defining factor in phytotoxicity. On the other hand, the concentrations measured in the mature compost were predominantly below the EC10 values.

The results of the thesis provide valuable information on the phytotoxicity of composts and can be used in evaluation of their phytotoxic potential originating from LWCA.

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YHTEENVETO (RÉSUMÉ IN FINNISH)

Lyhytketjuisten karboksyylihappojen rooli kompostin fytotoksisuudessa

Suomessa biojätteen kierrätys erilliskeräyksen ja kompostoinnin kautta on lisääntynyt huomattavasti viimeisten kahdenkymmenen vuoden aikana. Toiminnan alku- ja vakiintumisvaiheessa kompostia käytettiin pääsääntöisesti vanhojen kaatopaikkojen maisemoinnissa tai peitekerroksena toimivilla kaatopaikoilla, jolloin sen laadulla ei ollut huomattavaa merkitystä. Kuitenkin erityisesti viimeisten viiden vuoden aikana ovat lannoitteiden ja turpeen hinnat nousseet, minkä vuoksi kompostin merkitys on kasvanut. Nykyään suurin osa valmistuvasta kompostista käytetään maaparannusaineena tai mullan osakomponenttina maataloudessa tai maisemoinnissa. Sen myötä lopputuotteen oikea laatu on tullut yhä tärkeämmäksi asiaksi, sillä hyvälaatuinen kypsä komposti parantaa maaperän rakennetta humuksen ansiosta ja toimii kasvien ravinne- ja hivenainelähteenä. Vastaavasti huonolaatuinen tai raaka komposti voi olla kasveille toksinen ja vaurioittaa tai heikentää niiden kasvua. On tiedossa tapauksia, joissa huononlaatuinen komposti on hidastanut siemenien itämistä tai taimien kasvua pellossa ja aiheuttanut huomattavia taloudellisia vahinkoja.

Kompostin mahdollisesta fytotoksisuudesta on tiedetty jo kauan, kuitenkin ilmiön syyt tai mekanismit ovat jääneet epäselviksi. Aikaisemmissa tutkimuksissa on havaittu viitteitä siitä, että tietyt yhdisteet, kuten lyhytketjuiset rasvahapot (low-weight carboxylic acids, LWCA), ammoniakki, fenolit tai etyleenioksidi olisivat syinä raa'an kompostin fytotoksisuuteen. Tämän väitöstutkimuksen tavoite oli saada lisää tietoa LWCA-yhdisteiden roolista fytotoksisuudessa. LWCA:t – ts. muurahais-, etikka-, propaani-, butaani-, valeriaana- ja heksaanihappo – ovat sekä haihtuvia että veteen liukenevia yhdisteitä, joiden hiiliketjun pituus on 1 – 6 atomia. Mikrobiologisesti ne muodostuvat vähähappisissa (hypoksisssa) olosuhteissa orgaanisen aineen hajottaessa, vaikka kompostointi onkin lähtökohtaisesti aerobinen prosessi. Aerobisuuden aste voi kuitenkin vaihdella: suuren orgaanisen materiaalimäärän hajotessa voi massan sisälle muodostua vähähappisia alueita, joissa orgaaninen aines hajoaa epätäydellisesti ja tällöin muodostuu LWCA-yhdisteitä.

Väitöstyössä tutkittiin miten eri kompostointiprosessin parametrit vaikuttivat fytotoksisuuteen ja LWCA:n pitoisuuksiin. Kolmella kompostointikoesarjalla arvioitiin, miten syöte, ilmastus ja lisä-aineet vaikuttivat kompostin fytotoksisuuteen ja LWCA:n dynamiikkaan. Kompostointikokeet tehtiin pienimittakaavaisina kaupallisissa kotikompostoreissa (220 l, Biolan). Kompostointisarjassa 1 syötteenä käytettiin biojätettä sekä aerobisesti ja anaerobisesti prosessoitua jätevesilietettä, jotka sekoitettiin turpeeseen suhteessa 1/1 (v/v). Kompostointisarjassa 2 syötteenä oli biojäte, turve ja puuhake (suhteessa 6/3/1, v/v/v), joka ilmastettiin kahdella teholla: 0,5 ja 5 l min^{-1}. Kompostointisarjassa 3 syötteenä oli biojäte ja turve (suhteessa 1/1, v/v). Toiseen kompostoriin sekoitettiin savipohjaista lisä-ainetta, suhteessa 1 kg $(100\ kg)^{-1}$ biojätettä ja toiseen kompos-

toriin kalkkipohjaista lisä-ainetta suhteessa 1,75 kg (100 kg)$^{-1}$ kompostoituvaa massaa. Kompostointikokeet kestivät 52 – 63 viikkoa, jonka aikana massoja sekoitettiin 1 – 2 viikon välein ensimmäisten 12 – 16 viikon aikana. Sen jälkeen komposteja jälkikypsytettiin, jolloin massoja sekoitettiin kolmesta neljään viikon välein. Sekoituksen yhteydessä otettiin kompostinäytteitä analysointia varten. Prosessin etenemistä seurattiin mittaamalla lämpötilaa ja kaasujen pitoisuuksia (CO_2, O_2, CH_4 ja NH_3) kaasufaasissa. Näytteistä määritettiin LWCA-pitoisuudet käyttäen kahta uuttotapaa (vesi- ja alkaaliuutto), analysoitiin ammonium- ja nitraattitypin ja raskasmetallien pitoisuudet sekä mitattiin fytotoksisuus krassille (*Lepidium sativum*) itävyys- ja kasvukokeissa. LWCA-yhdisteet identifiointiin käyttämällä derivatisointitekniikkaa, jossa LWCA esteröitiin benzylbromiidilla ja muodostuneet bentsyylieetterit identifioitiin GC—FID avulla. Menetelmä mahdollisti myös muurahaishapon pitoisuuden mittaamisen, joka on erittäin haastavaa ilman derivatisointia, jos muita lyhytketjuisia rasvahappoja on seoksessa. Kompostointikoesarjojen lisäksi tutkittiin LWCA-yhdisteiden fytotoksisuutta annos-vaste kokeina käyttäen yksittäisiä happoja, niiden seoksia sekä lyhyt- (72 t) ja pitkäkestoisia (21 vrk) vasteita kasveille. Mittausten perusteella laskettiin LWCA-yhdisteiden toksisuusarvoja (effective concentration, EC) ja verrattiin eri happojen fytotoksisuuspotentiaalia sekä arvioitiin niiden seosvaikutuksia.

Kompostointikokeet osoittivat, että orgaanisen aineen epästabiilisuus vaikuttaa erityisesti alkuvaiheessa prosessin aktiivisuuteen. Näin ollen biojätteiden hajoaminen kompostoinnissa on aktiivisempaa kuin lietteiden hajoaminen, mikä ilmeni mm. korkeampina lämpötiloina ja hiilidioksidin pitoisuuksina. LWCA-yhdisteiden dynamiikka oli myös erilainen biojätteen ja lietteiden kompostoinnissa. Kaikissa syötteissä pitoisuudet olivat luokkaa 10 g kg^{-1} kuiva-ainetta. Lietteiden kompostoituessa pitoisuudet pienentyivät yli 80 prosenttia jo kahden ensimmäisen viikon aikana, kun taas biojätteen kompostoituessa niitä akkumuloitui jopa neljän viikon ajan, minkä jälkeen pitoisuudet tasaantuivat pienemmälle tasolle. Oletuksen vastaisesti korkeampia LWCA-pitoisuuksia muodostui voimakkaammin kuin vähemmän voimakkaasti ilmastoidussa kompostorissa. Kalkkipohjainen lisäaine, joka sisälsi mm. kalkkiperoksiidia (1 %), tehosti etiikkahapon muodostumista kokeen alussa ja nosti kompostimassan pH- arvoja. Savipohjaisen lisäaineen vaikutuksia mitattuihin parametreihin ei havaittu. Kaikki kokeet huomioitaessa kompostoitumisen aktiivisessa vaiheessa LWCA-pitoisuus vähentyi 60 – 99 % syötteen pitoisuuksista ja vakiintui tietylle tasolle. Näin kypsään kompostiin muodostui LWCA:n varasto, joka kuiva-ainetta kohti oli 100 – 800 mg kg^{-1} (vesiuutteessa) tai 800 – 1500 mg kg^{-1} (alkaliuutteessa). Analysoiduista LWCA-yhdisteistä etikkahappo oli vallitseva ja sitä kertyi enemmän kuin muurahais-, propaani- ja voihappoa, valeriaana- ja heksaanihapon pitoisuudet jäivät kvantitatiivisten raja-arvojen alle (n. 30 mg kg^{-1} ka). Yleensä etiikkahappo on akkumuloinut kompostiin, kun taas muurahais-, butaani- ja voihappojen dynamiikka oli samankaltainen, niiden pitoisuudet pääsääntöisesti laskivat alkupitoisuuksista kompostoinnin edetessä.

Kasvikokeissa todettiin raa'an kompostin fytotoksisuuden kestävän 2 – 24 viikkoa kompostointiasetelmista riippuen. Voimakkaampi ilmastus ja kalkkipitoinen lisäaine lyhensivät fytotoksisen jakson kestoa. Korrelaatioanalyysi osoitti melko suoraa käänteistä riippuvuutta ($\rho = -0.77$) kompostifytotoksisuuden ja LWCA-yhdisteiden pitoisuuden välillä. Yksittäisten LWCA-yhdisteiden fytotoksisuus suureni molekyylin hiiliketjun pidetessä niin, että muurahaishappo oli vähiten ja heksaanihappo eniten toksinen. Happojen seoskokeissa todettiin, että LWCA-yhdisteillä on summavaikutus, mikä tarkoittaa että happoseoksen kokonaistoksisuus riippuu kunkin hapon osapitoisuudesta. Kasvikokeiden tulosten perustella laskettiin myös EC10 arvot LWCA-seokselle, joka vaikuttavuustaso oletettiin turvalliseksi kasveille. Seoksella, jossa kaikki hapot olivat samassa moolisuhteessa, EC10-arvo oli noin 800 mg kg^{-1} kuiva-ainetta kohden. Jos seoksessa hapot esiintyvät eri suhteessa, seoksen EC10-arvo voidaan laskea yksittäisten happojen perusteella niiden EC10 arvojen käyttäen.

РЕЗЮМЕ (RÉSUMÉ IN RUSSIAN)

Раздельный сбор пищевых отходов и их утилизация путем компостирования значительно возросли за последние двадцать лет в Финляндии. На начальной стадии и стадии становления системы утилизации, компост использовали в основном в качестве покровного материала при рекультивации закрытых свалок твердых бытовых отходов или покровного слоя на работающих полигонах. На данных объектах качество компоста не имело большого значения. За последние пять лет, в связи с ростом цен на торф и минеральные удобрения, объекты утилизации компоста изменились. В настоящее время бóльшая часть получаемого компоста утилизируется в качестве мелиорантов нарушенных почв или составляющего компонента в почво-покровных субстратах, которые широко используются в сельском хозяйстве и при благоустройстве территорий. На данных объектах высокое качество компоста является одним из основных условий для использования материала. Компост высокого качества улучшает структуру почвы за счет содержания гумуса и является источником макро- и микроэлементов для растений. С дугой стороны, незрелый компост может быть токсичным для растений (фитотоксичным), т.е. замедлять прорастание семян и задерживать рост всходов. Известны случаи повреждения всходов после применения незрелого компоста на сельхозугодиях, что повлекло за собой значительные экономические убытки.

О фитотоксичности незрелого компоста известно давно, хотя причины и механизмы феномена до конца не изучены. В ранее опубликованных исследованиях основными причинами фитотоксичности указываются высокое содержание низкомолекулярных жирных кислот (НМЖК), катионы аммония, фенольные соединения и оксид этилена. Целью данной диссертационной работы было исследование роли НМЖК в фитотоксичности незрелого компоста. НМЖК (муравьиная, уксусная, пропионовая, масляная, валериановая и капроновая кислоты) – это группа насыщенных, органических кислот, в молекулярную цепочку которых входит от одного до шести атомов углерода. Они летучи и растворимы в воде. В процессе компостирования НМЖК образуются при неполном микробиологическом окислении органического вещества в участках с низким содержанием кислорода.

В задачи данного исследования входило изучение влияния таких параметров процесса, как природа компостируемого материала, интенсивность аэрации и минеральных добавок на динамику фитотоксичности и содержание НМЖК. Эксперименты проводились в компостерах объемом 220 л (Biolan, Финляндия). В эксперименте 1 в качестве компостируемого материала использовались пищевые отходы и илы, образующиеся при очистке сточных вод аэробным или анаэробным способом, которые смешивались с торфом в объемном соотношении 1/1. В

эксперименте 2 компостируемая смесь (пищевые отходы/торф/древесная щепа в обьемном соотношении 6/3/1) аэрировалась с интенсивностью 0,5 или 5 л мин$^{-1}$. В эксперименте 3 в компостируемую смесь (пищевые отходы/торф/ в обьемном соотношении 1/1) добавлялись коммерческие минеральные добавки в количествах, рекомендованных производителем. В одном компостере применялась добавка на основе глины в соотношении 1 кг на 100 кг отходов, а в другом добавка на основе извести в соотношении 1,75 кг на 100 кг компостируемой массы. Эксперименты длились от 52 до 63 недель, во время которых масса перемешивалась раз в 2–3 недели в течение первых 12–16 недель и на стадии дозревания раз в 3–4 недели. Мониторинг процесса осуществлялся путем измерения показателей температуры и содержания углекислого газа, кислорода, метана и аммониака в газовой фазе над компостом. Во время перемешивания отбирались пробы компоста, в которых позднее определялось содержание НМЖК в водном и щелочном экстракте, аммонийного и нитратного азота, тяжелых металлов и фитотоксичность. Для определения НМЖК применялась дериватизация с использованием бензолбромида с последующей идентификацией эфиров бензола НМЖК с помощью ГХ-ПИД. Данный метод позволил измерить содержание муравьиной кислоты, что обычно представляет сложность при анализах смеси НМЖК без дериватизации. Фитотоксичность компостов определялась в кратковременных (72 ч) и субхронических опытах (21 сут.) с использованием кресс-салата *Lepidium sativum* в качестве тест-обьекта. В дополнение к определению фитотоксичности компостов, были проведены опыты по определению фитотоксичности отдельных кислот и их смесей. На основе результатов опытов были рассчитаны средние эффективные концентрации (EC10, EC50 и EC90) для каждой кислоты и оценен тип их взаимодействия в смесях.

В экспериментах компостирования было отмечено, что нестабильность органического вещества является определяющим фактором активности процесса. Активность разложения пищевых отходов была выше, чем илов, что подтвердилось измерениями показаний температуры, концентрацией углекислого газа и динамикой содержания НМЖК. Концентрация НМЖК во всех исходных материалах была порядка 10 г кг$^{-1}$ сухого остатка. При компостировании илов содержание НМЖК уменьшилось на 80 % уже в течение первых двух недель. При компостировании пищевых отходов наблюдалась аккумуляция НМЖК с увеличением концентрации в 2–4 раза по сравнению с исходным уровнем в течение первых четырех недель, после чего концентрация снизилась и стабилизировалась на определенном уровне. Вопреки ожиданиям, аккумуляция НМЖК при компостировании с высокой интенсивностью аэрации была выше, чем с низкой. При использовании добавки на основе извести отмечалось почти 10-ти кратное увеличение концентрации уксусной кислоты и более высокие значения pH. При использовании добавки на основе глины значительных изменений на процесс

компостирования и динамику измеренных параметров отмечено не было. При оценке результатов всех экспериментов можно обобщить, что в процессе компостирования содержание НМЖК уменьшилось на 60-99 % по сравнению с начальной концентрацией, и сформировался буферный запас НМЖК, выраженный в сухом остатке на уровне 100–800 мг $кг^{-1}$ (в водном экстракте) или 800–1500 мг $кг^{-1}$ (в щелочном экстракте). Из анализируемых НМЖК концентрации уксусной кислоты доминировали над концентрациями муравьиной, пропионовой и масляной кислот, концентрации валериановой и капроновой кислот были ниже предела чувствительности (ок. 30 мг $кг^{-1}$ сух. ост.). В большинстве случаев рост общей концентрации НМЖК происходил за счет роста концентрации уксусной кислоты. Динамика муравьиной, пропионовой и масляной кислот была схожей, их концентрации постепенно снижались в процессе компостирования.

Результаты опытов по определению фитотоксичности компостов показали, что в зависимости от параметров экспериментов, период фитотоксичности длился от 2 до 24 недель. Длительность фитотоксичного периода значительно уменьшилась при увеличении интенсивности аэрации и применении добавки на основе извести. Корреляционный анализ не выявил статистически значимой зависимости между концентрациями НМЖК и фитотоксичностью компоста ($\rho = -0.77$), хотя в опытах с индивидуальными кислотами было продемонстрировано явное увеличение фитотоксичности с увеличением их концентраций. По результатам опытов с чистыми кислотами можно утверждать, что фитотоксичность НМЖК увеличивается с ростом молекулярной цепочки кислоты, т.е. муравьиная кислота (C_1) обладает самой низкой токсичностью, а капроновая (C_6) самой высокой. На основе опытов со смесями можно заключить, что НМЖК обладают суммарной токсичностью и в смеси эффекта синергизма или антагонизма не возникает. По результатам опытов для смеси НМЖК было рассчитано значение EC10, которое можно считать достаточно безопасным уровнем для растений. Для смеси, где каждая кислота представлена в равных молярных соотношениях EC10 является 800 мг $кг^{-1}$ сух. ост. Если кислоты содержатся в разных соотношениях, то значение EC10 смеси пропорционально концентрации каждой кислоты в отдельности и она рассчитывается с помощью полученных значений EC10 для чистых кислот.

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ORIGINAL PAPERS

I

COMPOSTING OF BIO-WASTE, AEROBIC AND ANAEROBIC SLUDGES – EFFECT OF FEEDSTOCK ON THE PROCESS AND QUALITY OF COMPOST

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Composting of bio-waste, aerobic and anaerobic sludges – Effect of feedstock on the process and quality of compost

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ABSTRACT

In-vessel composting of three stocks with originally different degree of organic matter degradation was conducted for: (1) kitchen source-separated bio-waste (BW), (2) aerobic (AS) as well as (3) anaerobic sludges (AnS) from municipal wastewater treatment plant. Composting experiment lasted over a year. The highest activity of the process was in the BW compost. It was implied by the highest temperature, CO_2 release, ammonification and nitrification, intensive accumulation and removal of low-weight carboxylic acids (water- and NaOH-extractable). Between the sludges higher mineralization and CO2 release was in AnS, while ammonification and nitrification were higher in AS compost; no significant difference between sludge composts was noticed for dynamics of pH, conductivity, concentrations of LWCA, and some nutrient compounds and heavy metals. Nitrogen content of the final compost increased in BW, but decreased in AS and AnS. Phytotoxicity of *Lepidium sativum* was eliminated faster in sludge composts compared to BW compost.

1. Introduction

Urban organic waste (food scrapes, green and park wastes, sewage sludge, etc.) is a very unstable quickly biodegrading waste fraction. Its treatment is challenging in terms of avoiding odour and pathogen nuisance. On the other hand it has high potential as soil amendment. According to Eurostat the estimated generation rate of animal and vegetable agricultural waste in EU countries in 2006 was 95 Mt. The estimated amount of animal waste generated from food production was 13 Mt, the amount of sewage sludge arising was about 17 Mt. Composting is regarded as a suitable way for recycling such type of waste because it helps to solve the problem of their disposal, reduce emissions of greenhouse gas, and also result in a useful soil improving agent – compost. This end product can be used for agricultural purposes to improve soils, and especially to recover the degraded soils in semiarid zones, because its incorporation in soil in suitable conditions increases fertility (Banegas et al., 2007).

According to the Green Paper on the Management of Bio-waste in EU (Commission of the European Communities, 2008) the total production of compost in 2005 in EU countries was 13.2 Mt. Most of it was produced from bio-waste (4.8 Mt) and green waste (5.7 Mt), the rest from sewage sludge (1.4 Mt) and mixed waste (1.4 Mt). The potential of compost production from most valuable inputs (bio-waste and green waste) is estimated at 35–40 Mt, which means that high quality compost can significantly improve quality of European soils. However, production of high quality compost is not necessarily the aim of waste-processing companies. As on one hand compost production process is financed by the gate fees for the waste, so the commercial success of compost production depends very little on the sale of the final product. On the other hand, in most European countries the quality of composts is defined by low contents of pollutants particularly heavy metals, organic pollutants, impurities (Binner et al., 2008), with no standards set for the quality as soil improver. Overall, bio-waste as source of nutrients is highly under used.

Although composting is a widely used process, there are still knowledge gaps in understanding it due to high variety and heterogeneity of the feedstock materials, abundance of the processing technologies, openness of the system, end product chemistry, etc. Studies have been conducted on different feedstocks like bio-waste (Pascual et al., 1997; Kirchman and Widén, 1994), aerobic and anaerobic sludges (Banegas et al., 2007; Fuentes et al., 2006; Li et al., 2001), and manures (Tiquia and Tam, 1998) with various bulking agents and additives. However, few attempts have been made to compare composting process of three most widely used feedstocks in one research to reveal effect of status of organic matter degradation on the process and on the quality of the final product. This is an important aspect because in general composting sums up to mineralization of organic matter and is highly driven by the availability of organic compounds for the microorganisms.

The aim of our research was to compare effectiveness of material stabilization during composting of three feedstocks with

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originally different status of organic matter degradation (kitchen bio-waste, aerobic and anaerobic sludges from municipal wastewater treatment plant) and assess the suitability of the final products as soil amendment.

2. Methods

2.1. Composting experiment

A laboratory trial was performed with three different feedstocks – at-source separated kitchen bio-waste (BW), aerobic sludge (AS), and de-watered municipal anaerobic sludge (AnS). Bio-waste was collected for 3 days at the food catering centre of the town of Mikkeli (Finland). It was a mixture of food leftovers and vegetable waste (i.e. salad stunts, cabbage leaves and stunts, carrot and potato peals, etc.). Aerobic sludge was de-watered product from the wastewater treatment facility in municipality of Ristiina close to Mikkeli. Anaerobic sludge was de-watered product from the third anaerobic stage after pre-treatment and aerobic treatment collected at the wastewater treatment plant in Mikkeli town. Both sludges were coming from low industrialized towns. Each feedstock was mixed with sphagnum peat as bulk material in proportion 1 + 1 (v/v). The peat was collected at local waste-processing company. Three insulated lid-covered composters (220 L, Biolan, Finland) supplied with leachate-collecting system were filled with three different feedstock-peat mixtures. Passive aeration of the composters occurred through a system of bored holes and was based on air-pressure difference between the inner and outer parts of the composter. Some characteristics of the mixtures are presented in Table 1.

The experiment lasted for 63 weeks. During this period the composting mass was mixed manually once every 2 weeks during the first 12 weeks and after that on week 16, 24 and 63. For mixing, all the material was removed from the composter into a separate container and thoroughly mixed with shovel, no water was added. After mixing, about 10 L of a composite sample was taken; one part of the sample was analyzed immediately, another part dried at 35 °C, and a third part frozen at −18 °C until processed for further analysis. After sampling, the material was put back into the composter.

Table 1
Characteristics of the materials at the beginning of composting experiment. BW = bio-waste + peat (1 + 1, v/v), AS = aerobic sludge + peat (1 + 1, v/v), and AnS = anaerobic sludge + peat (1 + 1, v/v). Mean ± SD of triplicate subsamples, except total low-weight carboxylic acids (LWCA), which are means of four replicate subsamples.

Characteristic	BW	AS	AnS
pH (compost-water mixture, 1 + 5, v/v)	4.5 ± 0.1	5.7 ± 0.0	6.4 ± 0.1
Conductivity (1 + 5, mS/cm)	0.69 ± 0.07	0.27 ± 0.02	0.88 ± 0.11
Dry matter (%)	31.4 ± 0.0	15.4 ± 0.0	16.0 ± 0.0
Ash content (%)	23.0 ± 3.3	19.2 ± 1.6	19.6 ± 0.2
Total C (%)	39.0 ± 0.6	36.5 ± 0.4	34.1 ± 0.6
Total N (%)	2.0 ± 0.1	2.4 ± 0.02	2.1 ± 0.03
C/N ratio	20	15	16
Total water-soluble LWCA[a] (mg/kg dw)	8600 ± 650	13,800 ± 800	11,200 ± 550
Total NaOH-soluble LWCA[a] (mg/kg dw)	12,300 ± 700	14,300 ± 1000	6600 ± 4600
Phytotoxicity, 48 h (% control)[b]	8.1 ± 2.1	6.6 ± 0.8	2.3 ± 1.9
Phytotoxicity, 14 days (% control)[c]	34.4 ± 9.7	56.6 ± 9.9	44.6 ± 11.1

[a] Low-weight carboxylic acids.
[b] Obtained after 48 h incubation of *L. sativum* in water extract of compost (1:2; v/v), control – de-ionized water.
[c] Obtained after 14 days growth of *Lepidium sativum* in compost – peat-based growth medium (1:2, v/v), control – peat-based growth medium.

2.2. Monitoring and compost analysis

The process was monitored by measuring the temperature of the compost at 50 cm depth and concentrations of gases inside the composter above the mass before mixing of the composts. Gas was pumped into gas-collecting bag using silicone hose put through the hole in the lid of the composter. An infrared gas analyzer (GA94, Geotechnical Instruments) was used for analysis of oxygen, carbon dioxide and methane, and Dräger detection tubes (0.25/a and 5/a, National Dräger Inc., USA) for analysis of gaseous ammonia. The pH and conductivity of fresh compost samples were analyzed according to standard methods CEN13037 and CEN13038, respectively (European Committee for Standardization, 1999a,b). The concentration of water-soluble ammonium-, nitrate- and nitrite-nitrogen in compost–distilled water extract (1 + 5, v/v) was detected using test strips (Merckoquant ammonium test and nitrate test; Merck Chemicals, Darmstadt, Germany); detection limit for NH_4–N was 7.8 mg/L, NO_2–N 0.6 mg/L, and NO_3–N 2.3 mg/L.

Dry matter (at 105 °C, 24 h) and ash content (at 550 °C, 24 h) were measured according to the modified standard CEN13039 (European Committee for Standardization, 1999c). Based on the results of ash measurements, the mineralization rate of organic matter was calculated based on ash conservation principle. According to the principle, while organic matter degrades the absolute amount of ash does not change until formation of nitrites and nitrates as a result of nitrification. In this case absolute ash content slightly increases, but this change is negligible compared to overall ash content. Based on this assumption, mineralization rate was calculated according to the formulas:

At the start of composting :

$$M_{\text{ash}} = M_{\text{compost }0} \times C_{\text{ash }0} \quad \text{and} \quad M_{\text{org }0} = M_{\text{compost }0} - M_{\text{ash}} \tag{1}$$

After t time of composting :

$$M_{\text{compost }0} \times C_{\text{ash }0}/C_{\text{ash }t} \quad \text{and} \quad M_{\text{org }t} = M_{\text{compost }t} - M_{\text{ash}} \tag{2}$$

$$\text{MR} = M_{\text{org }t}/M_{\text{org }0} \times 100, \tag{3}$$

where M_{ash} = mass of ash (kg), $M_{\text{compost }0}$ = total mass of compost in the beginning of composting (kg), $M_{\text{compost }t}$ = total mass of compost after t time of composting (kg), $C_{\text{ash }0,\,t}$ = percent of ash analyzed in compost mass in the beginning of composting (%), $C_{\text{ash }0,\,t}$ = percent of ash analyzed in compost mass after t time of composting (%), $M_{\text{org }0,\,t}$ = mass of organic matter in compost in the beginning or after t time of composting (kg), and MR = mineralization rate during t time of composting (%).

For calculations compost was weighted in the beginning of the experiment.

Concentration of macro- and microelements, and heavy metals were analyzed in compost samples taken as mentioned above after 1, 8 and 63 weeks of composting. Analyses were made at the Department of Chemistry in Jyväskylä University. Prior to the analysis the samples were dried at 35 °C for 48 h, milled and sieved through a 2 mm mesh. Carbon and nitrogen were measured with CHNOS elemental analyzer using a TCD detector after combustion at 800 °C (Elementar vario ELIII, Elementar Analysensystemem GmbH, Germany). Macro- and microelements and heavy metals were extracted with aqua-regia–water mixture (1:1, v/v) and ultrasound assistance, and analyzed with ICP-AES (Model 2000, PerkinElmer, USA) according to Väisänen et al. (2002). Samples were analyzed in triplicates.

Low-weight carboxylic acids (LWCA) were analyzed in compost-H_2O and compost NaOH (0.1 M) extracts according to modified method given in Alén at al. (1985). For detailed description of the sample's pre-treatment and method modifications see

Himanen and Hänninen (2009). Samples were analyzed in four replicates. Concentrations of acids are expressed as mg acid per kg of dry compost.

2.3. Phytotoxicity assays

The toxicity of composts to plants was tested with two methods – 48-h germination assay and 21-days plant growth assay. Germination assay is a quick and widely used method for evaluating compost phytotoxicity. However, it allows a rather short period of time to follow plant development. On the contrary, plant growth bioassay lasts longer, but it gives a better picture of compost impact on plant growth.

Germination assays were conducted on compost – de-ionized water extracts (1:1, v/v). For the extract preparation 300 mL of compost was mixed with 300 mL of de-ionized water, shaken for 1 h at 180 rpm, centrifuged at 5000 rpm for 15 min, filtered through pre-rinsed filter paper (Whatman no. 4, Ø15 cm), divided into batches of 40 ml and frozen at −20 °C until needed. Five milliliters of the extract, de-frozen at 4 °C and equilibrated at room temperature, were added to a Petri dish (Ø9 cm) lined with filter paper (Whatman no. 1, Ø7.5 cm); de-ionized water was used as control. Twenty seeds of garden cress (*Lepidium sativum L.*) were placed in each Petri dish, closed with a lid, and incubated in the darkness at 25–27 °C. After 48 h, germination and length of radicle (accuracy 1 mm) were measured. The seed was considered germinated when the radical was at least 1 mm long. The experiment was accepted as valid when germination in control was above 95%. Each plate had three replicates and an extract from each compost sample was tested five times. The compost was considered to be non-toxic, when no statistically significant difference ($p > 0.05$) between the parameter (germination or seedling length) in compost extract and in the control was measured.

Before the plant growth assays compost that has been stored at −18 °C was de-frozen slowly overnight at 4 °C and temperated at room temperature. The assays were conducted on mixtures of compost and a peat-based growing medium (PBGM) at a 1:2 (v/v) ratio. PBGM (Finnpeat SI400, Kekkilä, Finland) was used as a control. Plastic pots (vol. 400 ml) were filled with either PBGM-compost mixture or PBGM and 25 seeds of garden cress (*L. sativum* L.) were spread on the surface and covered with a small amount of PBGM. The pots were placed in a greenhouse at a temperature of 25–27 °C, humidity 60% and 16/8 h light/dark cycle and incubated for 21 days. The pots were irrigated with de-ionized water on demand. The number of germinated seeds that appeared above the substrate was counted on days 7 and 14. On day 14 the amount of seedlings was reduced to 15 and they were incubated for another 7 days. On the day of termination the seedlings were cut close to the substrate surface, dried at 70 °C and weighed (accuracy 10 mg). The compost was considered to be non-toxic, when no statistically significant difference ($p > 0.05$) between the parameter (germination or total shoot weight) of the compost and the control was measured.

2.4. Statistical analysis

Averages and standard deviations were calculated with MS Office Excel 2003. The statistical significance of phytotoxicity for seedling length and seedling weight was tested with *t*-test ($p < 0.05$) and for germination – with Bernoulli test using R (version 2.8.1), drc package. The correlation between phytotoxicity results and the other measured parameters (concentrations of LWCA, water-soluble NH_4–N and pH) is presented as bivariate square Pearson's correlation coefficients.

3. Results and discussion

3.1. Temperature and gas emissions

During the experiment composting process has fully gone through active and maturation phases. Thermophilic phase was reached within 1 day in BW compost (max. $T = 63$ °C) and lasted during weeks 1 and 4, during weeks 2 and 3 temperature was in mesophilic range (35–45 °C) (Fig. 1). Most likely temperature drop between weeks 2 and 3 happened as result of the mixing, during which significant amount of heat was lost and it took time to accumulate it again enough to reach thermophilic temperature. In AnS thermophilic temperature was reached within 4 days (max. $T = 57$ °C) and lasted for two first weeks. Instead, the AS compost operated at mesophilic temperatures during the whole experiment (max. $T = 44$ °C). Ambient temperatures in all three composters were reached by week 12. Similar temperature dynamics as in BW and AnS are reported in other publications with slight difference in duration of thermophilic phase and maximum temperatures (Spaggiari and Spigoni, 1987; Kirchmann and Widén, 1994; Lee et al., 2002; Himanen and Hänninen, 2009). Although composting of AS proceeded at mesophilic temperatures in this experiment, composting of AS at thermophilic conditions have been reported, too (García et al., 1991). In research of Banegas et al. (2007) AS composing operated at thermophilic temperatures, while AnS – at mesophilic. From the previous experience at Heinola WWTP (Finland), where AS sludge is firstly composted in drum composter and then in windrows, the temperature in windrows could rise so high that there was a danger of self-ignition. So, composting temperature appears to be rather case-specific parameter, as it may vary and do not necessarily depend only on a feedstock composition but on other parameters, too.

Basically, emissions of CO_2 indicate mineralization and full degradation of organic matter (Epstein, 1997). Although it is not possible to make complete comparison of different variants without knowing the throughput of air, however, sufficiently good approximation on mineralization may be done. The highest concentrations of CO_2 were detected in BW compost with the peak of 19 vol% on the 12th day of processing, followed by a long-lasting gradual decrease. Even after 3 months peaks up to 5 vol% were still registered (Fig. 1). Drops in CO_2 content after mixing of the mass can be explained by dilution of the emissions from the mass with atmospheric air that contains about 0.03 vol% of CO_2. The second highest emission level of 18 vol% was registered in AnS compost measured on the fifth day followed by rather quick drop during the following 2 weeks and gradual diminish during the next 2 months. Emissions of CO_2 from AS compost were the lowest with maximum of 12 vol% measured on the third day. By the end of the first week concentrations dropped below 2 vol% and stayed on that level for the rest of the experiment. Large differences between the bio-waste and the sludges in CO_2 emissions can be explained with different degree of organic matter degradation in feedstocks. Degradation of organics in BW mainly started at the beginning of the experiment, while sludges have gone through pre-treatment at wastewater facilities where the largest part of easily degradable original organic matter has been modified. Significant difference between sludges could possibly be explained by formation of the secondary organic matter with the chain of dominating fermentation-oxidation reactions in AnS composting while in AS composting they were more oxidation–oxidation types and therefore, less effective.

Emissions of ammonia were detected between weeks 1 and 8 with maximum values on week 5 (Fig. 1). During this period concentrations in BW ranged from 0.04 to 23 ppm, in AS from 1.20 to 48 ppm, and in AnS from 2 to 32 ppm. Small concentrations of methane (up to 0.02 vol%) were detected from time to time,

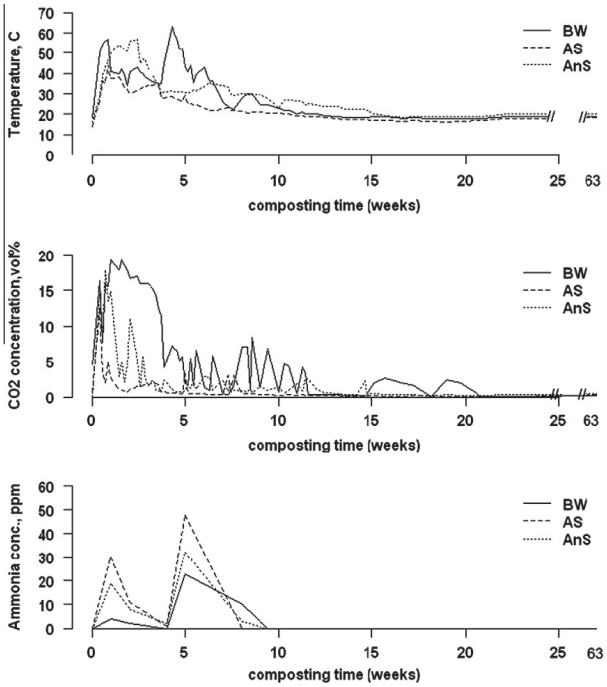

Fig. 1. Temperature, carbon dioxide, and ammonia concentrations measured in composts with different feedstock materials. BW – bio-waste, AS – aerobic sludge and AnS – anaerobic sludge. Concentrations of ammonia after week 8 were below detectable level (0.25 ppm).

however, mostly that was below detectable limit. Presumably, methane in small amounts was formed as result of aerobic oxidation reactions with similar mechanism of terpene carbohydrates formation during composting.

3.2. Compost characteristics

3.2.1. pH and conductivity

During the experiment pH in BW compost fluctuated between 4 and 5.5, with an exception on week 8 when pH was 8.55 (Fig. 2). Dynamics of pH in sludges was similar to each other: after the start around six it rose up to 7.5 already during the first week and stayed close to this level for the next 6 weeks, thereafter gradually decreasing to 3.5 by week 16. After 63 weeks of composting pH was about five in all three composts favorable for plant growth as at pH 5–6 most nutrients have maximum availability for plants (Johnston, 2003). Acidic pH in the beginning of bio-waste composting is rather typical changing to neutral or slightly basic as the process proceeds (Kirchmann and Widén, 1994; Lee et al., 2002; Himanen and Hänninen, 2009; Som et al., 2009). However, in this trial low pH during the whole trial could be possibly explained by using peat as bulking agent, which is acidic in nature and has high buffering capacity to prevent increase of pH to basic values. During composting of sludges pH usually changes from slightly basic to slightly acidic (Banegas et al., 2007; Li et al., 2001) that was observed in this experiment also.

Conductivity (EC) in BW compost was 2–3 times higher (range 0.1–1.4 mS/cm) than in the sludge composts (range 0.35–0.65 mS/cm) during the whole experiment (Fig. 2). High EC in BW compost can be explained by active degradation processes that led to release of ions bound to organic matter into water-soluble form. High conductivity might cause compost phytotoxicity as high concentrations of salts decrease osmotic pressure between plant roots and growth substrate and thus affect water availability to the plant (Bewley and Black, 1994). In spite of relatively high values of EC in BW compost, it was at an acceptable level in terms of safe applications for plant growth, according to ASCP Guidelines (2001) it should be below 2.5 mS/cm. EC of sludge composts were comparable with the EC dynamics reported in the other works (Li et al., 2001; Banegas et al., 2007).

In this trial dynamics of pH and EC were alike in the sludges and differ a lot from the bio-waste. Presumably degree of organic matter degradation and pre-treatment at wastewater facilities had strong influence on these parameters.

3.2.2. Dry matter, ash content and mineralization rate

During the experiment dry matter of BW compost was in a range of 25–40%. Dry matter in both sludge composts varied between 15% and 30%, which is rather low as recommended values should be in a range from 30% to 50% (McFarland, 2000), because high moisture content leads to smaller free air space that is disadvantageous for oxygen supply. In spite of high moisture content no free leachate was formed due to high water holding capacity of peat used as bulking agent.

Due to mineralization of organic matter relative ash content was increasing gradually, except for high peak on week 8 in AS

compost (Fig. 3). Net surplus of ash was about 12% (BW), 4% (AS) and 17% (AnS), which corresponds to 39%, 8% and 56% of the degraded organic matter (OM) (Fig. 3). During the experiment OM mineralization was intensive in BW right from the beginning,

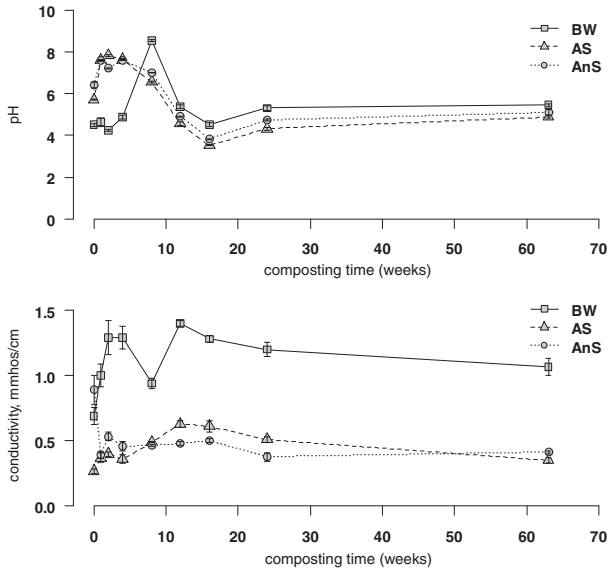

Fig. 2. Dynamics of pH and conductivity during composting of bio-waste (BW), aerobic (AS) and anaerobic (AnS) sludges. Error bars are standard deviations of triplicate determination.

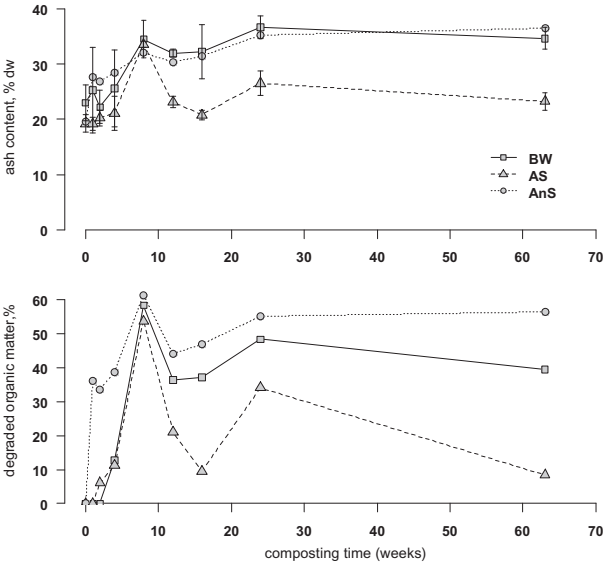

Fig. 3. Dynamics of ash content and mineralization during composting of bio-waste (BW), and aerobic (AS) and anaerobic sludges (AnS). Bars indicate standard deviation of triplicate determinations.

while in sludges there was a 4 weeks lag phase before mineralization could be recorded. Between the sludges mineralization was more successful in AnS than in AS. Compared to other works decrease in OM content in this study in AS was similar while mineralization in AnS was much higher. For example Banegas with co-workers (2007) recorded 12.7% decrease for 90 days composting of AS with wood sawdust (1:1), for anaerobic sludge mineralization it was 7.87%. Fang with co-workers (1999) reported a 9% OM loss during 100 days composting of AnS with sawdust amendment. However, it is difficult to compare results of different experiments due to different stabilization degrees of sludge, degradability of bulking agent and rotting conditions.

3.2.3. C/N ratio, ammonium- and nitrate-nitrogen

During over a year of composting the concentration of total nitrogen in BW compost increased from 2% to 2.6% and carbon decreased from 39% to 33.4% (Table 2). Thus, C/N ratio decreased from 20 to 13, which is typical for bio-waste composting process (Stratton and Rechcigl, 1998). On the contrary, in sludges both total carbon and total nitrogen decreased. In AS decrease for nitrogen was from 2.4% to 1.7% and for carbon from 36.5% to 34.2%; in AS decrease for nitrogen was from 2.1% to 1.7% and for carbon from 34.1% to 28.1%. Therefore, the C/N ratio increased from 15 to 20 in AS compost and stayed on the same level of 16 in AnS.

Table 2
Concentrations of total carbon and nitrogen the C/N ratio, and % of inorganic nitrogen in composts with different feedstocks and maturity level. BW – bio-waste, AS – aerobic sludge, and AnS – anaerobic sludge. Mean ± SD of triplicate subsamples cates.

	C	N	C/N	% of inorg. N	N loss started from wk 1	
	(%dw)	(%dw)	Ratio	(%total N)	(% N on wk1)	kg N/t dw
BW						
Week 1	39.0 ± 0.6	2.0 ± 0.1	20	4.5	–	–
Week 8	33.1 ± 1.5	2.5 ± 0.1	13	17.1	8.70	1.59
Week 63	33.4 ± 1.3	2.6 ± 0.1	13	16.6	5.32	0.97
AS						
Week 1	36.5 ± 0.4	2.4 ± 0.02	15	10.8	–	–
Week 8	34.1 ± 0.6	2.3 ± 0.03	15	25.7	45.24	1.09
Week 63	34.2 ± 0.4	1.7 ± 0.10	20	23.5	41.38	0.99
AnS						
Week 1	34.1 ± 0.6	2.1 ± 0.03	16	9.5	–	–
Week 8	31.4 ± 0.2	2.4 ± 0.04	13	29.7	1.43	0.02
Week 63	28.1 ± 0.9	1.7 ± 0.04	16	6.9	38.79	0.58

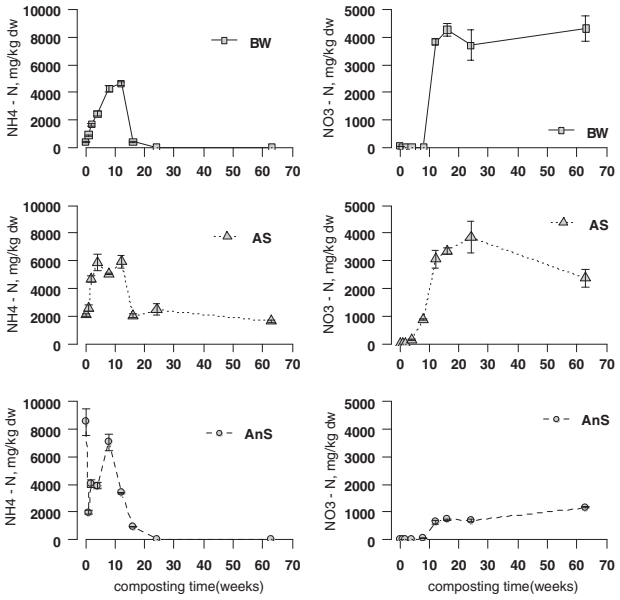

Fig. 4. Dynamics of water-soluble NH_4–N and NO_3–N during composting of bio-waste (BW), aerobic (AS) and anaerobic (AnS) sludges; mean ± SD measured in triplicate analysis.

Using data on ash content and total nitrogen measurements nitrogen losses were estimated (Table 2). During the trial total nitrogen loss was significant in sludges (about 40%) and smaller in BW (about 5%). As no free leachate was formed loss of nitrogen in liquid form did not occur and it happened through volatilization of ammonia. Low C/N ratio and high pH favoured stripping of gaseous ammonia from sludge composts that was monitored and presented in Fig. 1.

Ammonification was strong in BW and AS composts (Fig. 4). During the first 12 weeks the amount of soluble NH_4–N increased from 300 to 4500 mg/kg dw in BW and from 2200 to 6000 mg/kg dw in AS. Concentrations decreased rapidly between weeks 12 and 16, and reached below detectable limit by week 24 in BW, but was still 1400 mg/kg dw in AS compost at the end of the experiment. In AnS initial concentration of soluble NH_4–N was high (8500 mg/kg dw) but decreased more than half between weeks 1 and 2 due to stripping of gaseous ammonia. The ammonification peaked again on week 8 reaching 7000 mg/kg dw, by week 24 concentration of NH_4–N was below detectable limit.

Nitrification was effective in BW compost, concentration of soluble NO_3–N increased significantly between weeks 8 and 12 (from below detectable limit to 4000 mg/kg dw) and stayed around this level till the end. In AS concentration of NO_3–N was increasing between weeks 4 and 24 reaching its maximum of 3800 mg/kg and after that dropped to 2300 mg/kg dw by the end of the experiment. Nitrification in AnS was rather weak, concentration of NO_3–N was gradually increasing after 8 weeks of composting reaching maximum level of 1400 mg/kg dw by the end of the trial. Transformation of nitrogen is rather complicated process depending simultaneously on many aspects like pH, temperature, C/N ratio in the feedstock (Bernal et al., 2009). Similar dynamics of NH_4–N and NO_3–N during composting of bio-waste and sludges were observed by other authors (Kirchmann and Widén, 1994; Fang et al., 1999; Banegas et al., 2007; Himanen and Hänninen, 2009). According to the decree of Ministry of agriculture and forestry of Finland on fertilizers (656/01/2007) compost is suitable for application as soil amendment if NO_3–N/NH_4–N ratio is above 1. If compost fulfils this condition it means that ammonium concentration is low enough to cause compost phytotoxicity as well as it is rich in nitrates to support plant growth. By the end of the trial all composts fulfilled this criterion as ratio in BW was 4.3, in AS 1.5 and in AnS 1.2 with BW having better quality than the sludges.

3.2.4. Nutrients and heavy metals

Concentrations of most macro- and micronutrients in BW compost were similar or slightly higher than presented in previous works (Pascual et al., 1997; Himanen and Hänninen, 2009) (Table 3). Compost of both types of sludges contained high concentrations of iron and phosphorus, amount of other macro- and micronutrients were lower or on the same level compared to other

Table 4
Concentration of heavy metals (mg/kg dw) in bio-waste (BW), and aerobic (AS) and anaerobic (AnS) sludges during composting experiment (average and standard deviation of triplicate analysis) and maximum threshold values for heavy metals allowed in organic fertilizers according to the decree of Ministry of agriculture and forestry of Finland on fertilizers (656/01/2007).

	Cd	Cr	Cu	Ni	Pb	Zn
BW						
Week 1	<0.3[a]	<0.3	18.5	<2.6	18.8	203
SD			2.0		0.8	20
Week 8	<0.3	<3.0	14.3	2.9	12.8	208
SD			1.7	0.1	1.1	20
Week 63	<0.3	<3.0	18.3	3.6	17.0	217
SD			1.0	0.2	0.7	11
AS						
Week 1	<0.3	<3.0	108.1	9.3	53.5	350
SD			0.7	0.4	2.1	17
Week 8	<0.3	<3.0	135.0	10.4	70.3	380
SD			11.5	0.2	4.8	10
Week 63	<0.3	3.7	140.4	10.2	81.8	377
SD		1.0	19.9	1.6	17.9	33
AnS						
Week 1	<0.3	21.6	102.3	12.8	74.5	394
SD		1.3	5.1	0.3	7.3	30
Week 8	<0.3	24.9	92.0	13.0	83.3	414
SD		0.9	1.3	0.8	5.0	12
Week 63	<0.3	30.6	120.7	14.3	99.2	414
SD		2.2	8.7	0.6	2.3	10
Decree 656/01/2007	1.5	300	600	100	100	1500

[a] Below limit of detection.

Table 3
Concentration (mg/kg dw) of macro- and micronutrients during composting of bio-waste (BW), aerobic (AS) and anaerobic (AnS) sludges; Mean ± SD of triplicate subsamples.

	Al	Ca	Co	Fe	K	Na	Mg	Mn	Mo	P	S
BW											
Week 1	6200	8100	1.4	3650	5500	4900	2900	150	1.1	2200	1200
SD	250	400	0.1	30	500	150	70	20	0.1	30	100
Week 8	4700	15,500	1.7	3600	6700	6900	3300	200	1.6	3300	2300
SD	400	750	0.2	150	550	350	100	20	0.1	150	100
Week 63	5900	17,400	2.5	4700	8000	6300	3400	200	1.8	3700	3000
SD	200	150	0.1	100	250	300	30	20	0.1	40	50
AS											
Week 1	2700	4700	5.5	50,000	1300	1200	900	150	<0.9[a]	11,800	2000
SD	20	50	0.1	900	70	120	10	20		80	80
Week 8	3300	5700	6.4	60,000	1400	1100	1100	200	<0.9[a]	14,500	2700
SD	30	120	0.2	4300	60	100	30	40		350	450
Week 63	3600	6000	6.3	65,000	2100	1400	1100	230	<0.9[a]	16,300	3200
SD	450	700	2.5	6500	400	150	120	60		2300	700
AnS											
Week 1	4600	9500	6.0	60,700	1200	1200	1300	200	2.7	18,400	3500
SD	90	200	0.3	4500	200	100	30	20	0.2	650	250
Week 8	4700	10,500	5.8	64,600	1700	1400	1700	200	1.6	19,200	3600
SD	90	60	0.1	2200	250	10	30	4	0.3	250	150
Week 63	5600	10,000	6.9	70,100	1800	1300	1500	220	2.9	23,200	4400
SD	100	200	0.1	30	150	30	40	20	0.1	450	250

[a] Below limit of detection.

studies (Pascual et al., 1997; Fuentes et al., 2006). Elevated concentrations of iron can be explained with iron-based chemical agent that is used for precipitation of the sludge before drying at wastewater treatment facilities. High concentration of phosphorus is due to precipitation of the compound in solid fraction during wastewater treatment. Due to mineralization of organic matter during over a year of composting concentrations of macro-nutrients increased 20–50%.

Concentrations of heavy metals (Table 4) in composts were below or comparable with the concentrations presented in other studies (Pascual et al., 1997; Fang et al., 1999; Fuentes et al., 2006; Smith, 2009). In general, during 63 weeks of composting concentrations increased by 0–30%. However, neither initial nor final concentrations exceeded limit values allowed by decree of Ministry of agriculture and forestry of Finland on fertilizers (656/01/2007).

3.2.5. Dynamics of low-weight carboxylic acids

Low-weight carboxylic acids (LWCA) is a product of microbial organic matter degradation. During composting the acids can accumulate in compost causing bad odours of compost and being the reason for compost toxicity to plants (DeVleeschauwer et al., 1982; Brinton and Tränkner, 1999). For these reasons dynamics of acids were followed in this research.

Dynamics of LWCA in both sludges was similar to each other and was significantly different from the BW. Maximum concentrations of LWCA were two times higher in BW than in AS or AnS. By using two eluents (water and NaOH) it was possible to get a better picture on the acids dynamics during composting. In sludge composts concentration of acids, both water- and NaOH-extractable, were the highest in the beginning of the experiment, in BW compost the peak was on week 4 (Fig. 5). In BW from initial concentration of 8600 mg/kg dw (water-soluble) and 12,350 mg/kg dw (Na–OH soluble) amounts slightly dropped during the first week, but started to grow on week 2 reaching maximum on week 4 (water-soluble 27,100 mg/kg dw, NaOH-soluble 27,700 mg/kg dw). Already by week 8 concentrations dropped significantly to the levels they fluctuated around till the end of the trial (water-soluble 300–600 mg/kg dw and NaOH-soluble 700–1400 mg/kg dw). In AS compost initial total concentration of water-extractable acids was 13,800 mg/kg dw and NaOH-extractable 14,350 mg/kg dw; in AnS concentrations were 11,200 mg/kg dw and 6650 mg/kg dw, respectively. Amount of LWCA decreased significantly during the first 2 weeks of composting and thereafter fluctuated around that level till the end. In AS fluctuation was between 200–800 mg/kg dw for water-soluble and 1300–2000 mg/kg dw for Na–OH soluble acids; in AnS it was between 200–400 mg/kg dw and 900–1400 mg/kg dw, respectively. In the sludge composting, acids entered the process with the feedstock and then degraded as the process proceeded, which is rather typical for composting in general (Chanyasak et al., 1982; DeVleeschauwer et al., 1982; Himanen and Hänninen, 2009). On the contrary, a net surplus of the acids

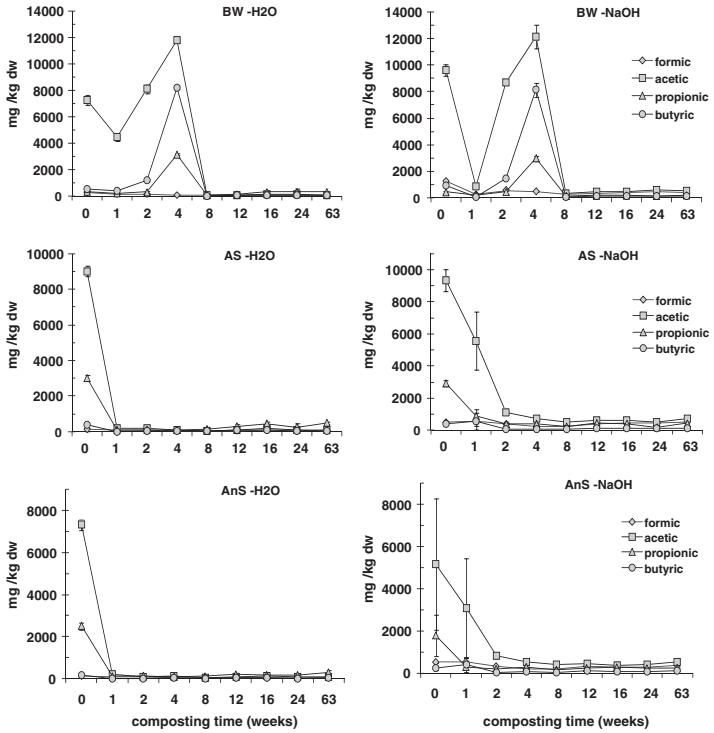

Fig. 5. Dynamics of formic, acetic, propionic, and butyric acids extracted with H_2O or NaOH from compost samples of different feedstock and age. Feedstock BW – bio-waste, AS – aerobic sludge and AnS – anaerobic sludge. mean ± SD measured in four replicate analysis.

measured on weeks 2 and 4 in BW compost indicated high microbial activity and degradation of easily degradable compounds that led to a temperature peak after week 4 (Fig. 1).

In the beginning of the experiment in sludge composts almost 100% of acids were water-extractable, but after 1 week of composting the situation changed, so only 10–50% of the acids could be extracted with water. In BW compost, during the first 4 weeks the acids were as water-extractable as NaOH-extractable, while from the week 8 onwards only 30–50% of the acid extracted with NaOH could be extracted with water also. Baziramakenga and Simard (1998) reported that around 34% of the acids extracted with NaOH from manure compost was water-extractable, whereas Himanen and Hänninen (2009) showed that 10–40% of acids in bio-waste compost were water-extractable. So, during active stage of composting when acids are constantly formed and degraded by microorganisms, they are more water-soluble and easily available for further microbial transformations. As the process proceeds formation of the acids decrease and also they presumably become attached to compost matrix forming an acid-pool in compost and may become water-soluble if compost becomes destabilized.

In all three composts dominating LWCA was acetic acid followed by butyric, propionic and formic acids in BW or propionic, formic, and butyric in sludge composts. Propionic acid was more water-extractable compared to other acids. Concentrations of iso-butyric, iso-valeric, valeric and caproic acids were detectable in the beginning of the process in all three composts but have dropped below detectable limits (50 mg/kg dw) very soon after beginning of the trial, therefore they are not presented in Fig. 5. Domination of acetic acid over the other LWCA has been noted in other works (Chanyasak et al., 1982; Kirchmann and Widén, 1994; Lee et al., 2002; Himanen and Hänninen, 2009). It can be explained by its role as common intermediate product during degradation of diverse substrates (Schlegel, 1988), its abundance indicating high microbial activity.

LWCA are essential compounds in formation of bad odours during composting. Odour nuisance is dependant not only on acid concentrations, but also on the type of the acid (Lechner and Binner, 1995) and interaction with other volatile organic compounds, e.g. like terpenes. So, bad odour is not a linearly scalable parameter, which means that small concentrations would be considered as bad but tolerable smell, but when concentrations increase two times the odour would be already unbearable. This is an important aspect that should be taken into consideration when increasing the size of composting plant or moving from pilot experiments to a full-scale plant. Odour nuisance especially in active phase of BW composting was a surprising aspect when composting of separated bio-waste was introduced in Finland, although there has been a lot of experience in composting of sewage sludge.

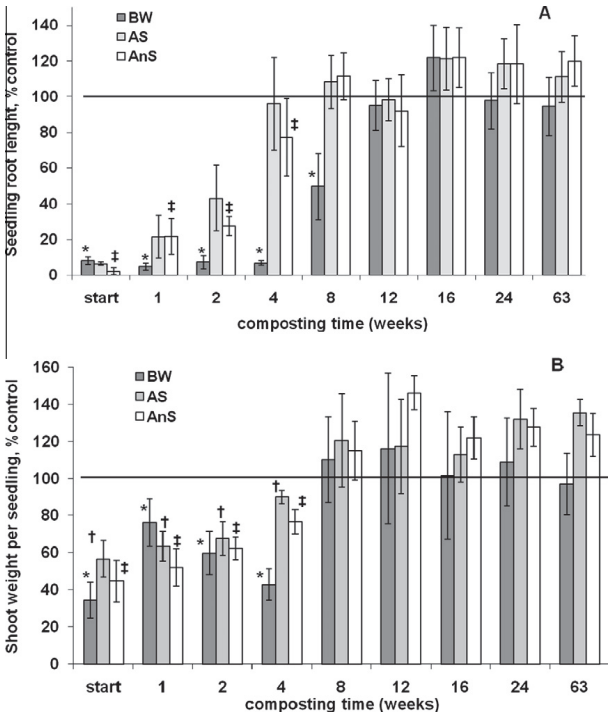

Fig. 6. Dynamics of phytotoxicity during composting of three feedstocks, with species *Lepidium sativum*. BW – bio-waste compost, AS – aerobic sludge, AnS – anaerobic sludge. Results of germination bioassay (A) are expressed as ratio of root length of seedlings in compost extract and control (de-ionized water); mean ± SD of five replications. Results of plant growth bioassay (B) are expressed as ratio of shoot weight in compost and control (peat-based growing media); mean ± SD, two replications. Black line at 100% indicates performance of the parameter in control. Symbols (*,†, ‡) indicate statistically difference ($p < 0.05$) between the control and the compost.

Table 5
Square Pearson's correlation coefficients of two phytotoxicity parameters to concentrations of acids (formic, acetic, propionic, butyric, and total) extracted with H_2O and NaOH, NH_4–N, and pH through time. Tested species – *Lepidium sativum*. Substrates studied in bioassays: water extracts (A) or mixture with peat-based growing medium (B) of bio-waste (BW), aerobic sludge (AS) or anaerobic sludge (AnS).

	Formic		Acetic		Propionic		Butyric		Total		NH_4–N	pH
	H_2O	NaOH	H_2O	NaOH	H_2O	NaOH	H_2O	NaOH	H_2O	NaOH		
(A) Root length per seedling												
BW	0.42	0.12	0.70	0.52	0.13	0.20	0.21	0.21	0.45	0.40	0.01	0.03
AS	0.43	0.19	0.40	0.74	0.29	0.55	0.26	0.55	0.38	0.71	0.00	0.32
AnS	0.79	0.49	0.36	0.66	0.31	0.36	0.29	0.37	0.39	0.66	0.56	0.42
(B) Dry shoot weight												
BW	0.50	0.45	0.86	0.84	0.28	0.34	0.33	0.36	0.63	0.67	0.06	0.26
AS	0.36	0.05	0.28	0.55	0.17	0.41	0.18	0.38	0.26	0.52	0.04	0.43
AnS	0.65	0.31	0.78	0.56	0.22	0.28	0.22	0.32	0.31	0.55	0.50	0.56

3.3. Phytotoxicity

Germination and plant growth bioassays showed toxicity of immature compost, with slight differences in duration of the phytotoxic period. Two parameters measured in both tests (germination and seedling length/shoots weight) were affected in a different way in compost of different age. In 48-h bioassay toxicity for seed germination was eliminated after 8 weeks of composting in all three composts. For seedling length no difference between compost and control ($p > 0.05$) was observed after 4 weeks in AS, after 8 weeks in AnS, and after 12 weeks in BW (Fig. 6). In 21-days plant growth test over 95% of seeds germinated in control germinated in samples of AS and AnS older than 4 weeks and in BW older than 12 weeks. For shoot mass no difference between control and composts was observed after 8 weeks of composting in all three feedstocks. So, degree of organic matter degradation has an impact on compost toxicity to some extent.

Reasons for immature compost toxicity, suggested in the literature include high concentrations of volatile organic acids (DeVleeschauwer et al., 1982; Shiralipour and McConnell, 1997; Brinton and Tränkner, 1999), high concentration of NH_4–N (Tiquia and Tam, 1998; Britto and Kronzucker, 2002), oxygen depletion (Brinton and Evans, 2002) or presence of heavy metals (Epstein, 1997). Correlation coefficients of phytotoxicity results to various physical and chemical parameters are presented in Table 5. Correlation between toxicity parameters and LWCA concentrations were not very high, ranging between 0.21 and 0.79, however, higher than for NH_4–N or pH. The possibility of toxicity due to heavy metals is rather small as concentrations of the substances are low (Table 4) and it has been reported that metal availability to plants reduce with maturation time (Smith, 2009). So, the reason for immature compost phytotoxicity may be something else but the studied parameters.

4. Conclusions

The experiment was aimed at studying role of organic matter degradation status on composting process and characteristics of the final product. In the experiment with bio-waste, and aerobic and anaerobic sludges, the highest activity was in BW compost. It was implied by higher temperature, CO_2 release, ammonification and nitrification, increase in total nitrogen, and intensive dynamics of LWCA. In terms of mineralization, composting of sludges was not as consistent. Higher mineralization and carbon dioxide release were recorded during AnS composting, while ammonification and nitrification were higher in AS compost. Phytotoxicity was eliminated earlier in sludge composts compared to BW compost. So, the less feedstock is processed the higher degradation activity would be during composting, however, characteristics needed for improvement of the plant growth are not only dependent on the status of degraded organic matter.

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II

EFFECT OF COMMERCIAL MINERAL-BASED ADDITIVES ON COMPOSTING AND COMPOST QUALITY

by

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Effect of commercial mineral-based additives on composting and compost quality

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ABSTRACT

The effectiveness of two commercial additives meant to improve the composting process was studied in a laboratory-scale experiment. Improver A (sulphates and oxides of iron, magnesium, manganese, and zinc mixed with clay) and B (mixture of calcium hydroxide, peroxide, and oxide) were added to source-separated biowaste:peat mixture (1:1, v/v) in proportions recommended by the producers. The composting process (*T*, emissions of CO_2, NH_3, and CH_4) and the quality of the compost (pH, conductivity, C/N ratio, water-soluble NH_4–N and NO_3–N, water- and NaOH-soluble low-weight carboxylic acids, nutrients, heavy metals and phytotoxicity to *Lepidium sarivum*) were monitored during one year. Compared with the control, the addition of improver B increased pH by two units, led to an earlier elimination of water-soluble ammonia, an increase in nitrates, a 10-fold increase in concentrations of acetic acid, and shortened phytotoxicity period by half; as negative aspect it led to volatilization of ammonia. The addition of improver A led to a longer thermophilic stage by one week and lower concentrations of low-weight carboxylic acids (both water- and NaOH-extractable) with formic and acetic of similar amounts, however, most of the aspects claimed by the improver's producer were not confirmed in this trial.

1. Introduction

As a response to the EU Council Directive on landfill of waste (1999/31/EC), the separation of kitchen biowaste has become a common practice in Finland. In 2006 the amount of biowaste collected at-source was 197,000 tonnes, 82% of which was utilized (Statistics Finland Agency, 2006). The most common way to utilize separated biowaste is composting, either on household properties or at large-scale composting plants. The popularity of composting led to a high demand on the market for composters of various scales as well as compost-related products, such as bulking materials and compost accelerators that are supposed to improve the process and the quality of compost.

Different market names for compost additives exist (e.g. compost accelerator, elixir, incentive, and starter) and they all sound very enticing and attractive to a customer. Usually, compost additives are mixtures of different amounts of various microorganisms, mineral nutrients or readily available forms of carbon, enzymes, and pH-balancing compounds that are meant to enhance microbial activity when the additive is in contact with the waste material. Instructions for additives provided by manufacturers may contain an approximate list of materials, a message promising that composting will be better and quicker; and the compost of a much higher quality than without additives. Of course, there is a price for using such additives. In 2006, prices for commercial improvers on the Finnish market varied from €10 to €50 per cubic meter of compostable biowaste. Razvi and Kramer (1996) reported that in the USA the additional costs of commercial activators varied from $1.8 to $12.3 per cubic meter of compostable grass clippings.

A search with Google® using the key word "compost accelerator" gave hundreds of results that offered compost improvers. Dozens of patents were found in the Esp@cenet database of the European Patent Office (2007), all claiming the positive impacts of different mixtures on the composting process. In scientific literature, data on the effectiveness of compost additives is scarce. Razvi and Kramer (1996) presented the results of an experiment, where they studied the effectiveness of seven commercial activators, top soil, and mature compost on the composting of grass clippings. They showed that commercial activators were not more effective than naturally available top soil or mature compost. Korhonen (2006) studied five accelerators commercially available in Finland by testing their effectiveness on composting a mixture of standard waste (potato 80%, bread 15%, and chicken feed 5%) with wood waste as bulking agent (1:1.5 v/v); and showed that none had a positive effect on temperature, nutrient dynamics or plant toxicity. On the other hand, some positive results were found for the addition of alkaline products to compost, e.g. ash or lime. Lau et al. (2001) reported that the addition of 10% of fly ash had a positive effect on seed germination and reduced the availability of heavy metals. However, it increased electrical conductivity and volatilization of ammonia, while decreasing the bioavailability of phosphorous. Koivula et al. (2004) studied the effect of bottom ash on the composting of source-separated catering waste and found that a 10% and 20% ash addition improved the temperature regime, mineralization and humification rates, and decreased loss

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of total nitrogen. Out of three lime addition rates to sewage sludge (0.63%, 1%, and 1.63% dw) tested by Wong and Fang (2000) the most efficient was 0.63%. This amount had a positive effect on composting by increasing temperature and CO_2 evolution without any negative effects on the microbial community.

We studied the effectiveness of two commercial mineral additives that are used for biowaste treatment, in particular their effect on the composting process (duration of active phase and gas emissions) and quality of the final product (e.g. content of nutrients and heavy metals, concentrations of low-weight organic acids, and phytotoxicity). The choice of additives was based on the fact that both additives have been developed and manufactured in Finland, while many others existing on the market are imported.

2. Materials and methods

2.1. Composting experiment

A laboratory trial with two commercially available mineral compost additives was conducted. Composting was carried out in insulated lid-covered composters (220 L) supplied with a leachate collection system. Aeration of the composters occurred through a system of bored holes and was based on air-pressure difference between the inner and outer parts of the composter (Biolan, Finland). No forced aeration was applied. Such composters are widely used by households on their properties. Three composters were filled with the following mixtures: at-source-separated kitchen biowaste and peat (1:1, v/v) (BW), biowaste–peat mixture and the additive A in proportion 1 kg/100 kg biowaste (BW + A), and biowaste–peat mixture and the additive B in proportion 1.75 kg/100 kg compost mass (BW + B). Peat and at-source-separated biowaste were collected at the Mustankorkea waste-processing company (Jyväskylä, Finland) where material was stored outdoors. According to the manufacturer of additive A, this is a mixture of zeolite or kaolin clay (70%), Mn_2SO_4 (10%), dolomite chalk (15%), ashes (5%), and Co_2SO_4 (<0.3%). The additive is sold under the name of GrowHow Komposti Eliksiiri, formerly Biodeg, and is claimed to accelerate the composting process, quickly stabilize pH, and speed up the humification process (European Patent Office, 2004). Additive B is a mixture of $Ca(OH)_2$ (>90%), CaO_2 (>6%), and CaO (<1%), which is manufactured by Nordkalk Oyj Abp and is sold under commercial name of Velox. The additive is claimed to accelerate the composting process, remove malodours and disinfect waste (Nordkalk, 2004). The dosage of additives was chosen according to manufacturers' recommendations. Some characteristics of the mixtures are presented in Table 1. The trial started in winter (January), therefore the biowaste was frozen.

The trial lasted for 52 weeks. During this period the composting mass was mixed manually four times during the first month, once a month during the next five months, and once every three months during the next six months. For mixing, all the material was removed from the composter into a separate container and thoroughly mixed with shovel. After mixing, about 10 L of a composite sample was taken; a part of the sample analyzed immediately, part dried at 35 °C, and a part frozen at −18 °C until needed. After sampling, the material was put back into the composter.

2.2. Monitoring and compost analysis

The process was monitored by measuring the temperature of the compost at 50 cm depth and the concentrations of gases inside the composter above the mass using an infrared gas analyzer for oxygen, carbon dioxide and methane (GA94, Geotechnical Instruments) or Dräger detection tubes for gaseous ammonia (2/a and 5/a, National Dräger Inc., USA). Due to technical problems, CO_2 was not measured during the second week of the trial. The conductivity and pH of fresh compost samples were analyzed according to standard methods CEN13037 and CEN13038, respectively (European Committee for Standardization, 1999a,b). A concentration of water-soluble ammonium-, nitrate- and nitrite-nitrogen in compost-water extract (1 + 5, v/v) was detected using test strips (Merckoquant ammonium test and nitrate test; Merck Chemicals, Darmstadt, Germany), detection limit for NH_4–N – 7.8 mg/L, NO_2–N – 0.6 mg/L, and NO_3–N – 2.3 mg/L.

Dry matter (at 105 °C until constant weight) and ash content (at 450 °C until constant weight) were measured according to the standard CEN13039 (European Committee for Standardization, 1999c). Based on the results of ash measurements, the mineralization rate of organic matter was calculated. The calculation is based on the assumption that, as composting proceeds and organic matter degrades, the absolute amount of ash does not change until nitrites and nitrates start to form as a result of nitrification. In this case absolute ash content slightly increases, but this change is negligible. Based on this assumption, mineralization rate was calculated assuming a constant ash content.

Concentrations of macro- and microelements, and heavy metals in compost samples taken as mentioned above after 0 (start), 12 and 24 weeks of composting were analyzed at the Geological Survey of Finland. The number of analyzed samples was limited by the project budget; therefore samples after 52 weeks of composting were not analyzed. Prior to analysis the samples were dried at 35 °C for 48 h, milled and sieved through a 2 mm mesh. Carbon and nitrogen were measured with a CN analyzer using a TC detec-

Table 1
Characteristics of the material in the beginning of composting trial. BW – biowaste:peat (1:1, v/v), BW + A – biowaste:peat + additive A, and BW + B – biowaste:peat + additive B; mean ± SD of triplicates, except total C and N, which are means of duplicates.

Characteristic	BW	BW + A	BW + B
pH (1 + 5)	4.52 ± 0.02	4.38 ± 0.05	6.99 ± 0.12
Conductivity (1 + 5, mmhos/cm)	230 ± 15	460 ± 40	880 ± 75
Dry matter (%)	40.6 ± 0.4	40.2 ± 0.07	38.6 ± 0.78
Ash content (%)	4.8 ± 0.6	8.8 ± 0.4	7.0 ± 0.3
Total C (%)	48.9	47.9	46.2
Total N (%)	1.55	1.80	1.65
C/N ratio	32	27	28
Total water-soluble LWCA[a] (mg/kg dw)	1000 ± 300	470 ± 180	10,600 ± 1050
Total NaOH-soluble LWCA[a] (mg/kg dw)	4000 ± 460	2850 ± 550	11,500 ± 930
Seedling length (% control)[b]	8.4 ± 4.5	9.1 ± 3.4	10.7 ± 1.9
Dry weight of seedling (% control)[c]	38.4 ± 6.9	50.4 ± 30.2	51.1 ± 6.9

[a] Low-weight carboxylic acids.
[b] Obtained after 48 h incubation of *L. sativum* in compost – water extract (1:1; v/v), control – de-ionized water.
[c] Obtained after 14 days growth of *L. sativum* in compost – peat-based growth medium (1:1; v/v), control – peat-based growth medium.

tor after combustion (Elementar Vario MAX C7N, Elementar Analysensysteme GmbH, Germany). Samples for macro- and microelements and heavy metals were digested with nitric acid in a microwave digester (MARS, CEM Corp., USA) according to standard method EPA3051 (US EPA, 1998) and then analyzed using ICP-AES technique (Thermo Jarrell ASH IRIS/Duo Wiev-ICP-OES, Thermo Jarrell Corp., USA).

Low-weight carboxylic acids (LWCA) were analyzed in compost-H_2O and compost-NaOH (0.1 M) extracts. By using mild alkaline extractant, organic acids that are weakly absorbed to the organic matrix desorb into the solution and can be analyzed. Extracts (2:3, v/v) were prepared by weighing 40 mL of compost (de-frozen at 4 °C) and mixing it with 60 mL of distilled H_2O or NaOH (0.1 M). The mixture was shaken for 1 h at 180 rpm (Heidolph unimax, 2010), centrifuged for 10 min at 6000 rpm and filtered (Whatman no. 4, Ø150 mm) to a 100 mL measuring bottle. After filtration crotonic acid was added as internal standard (3 mL, 7.5 mg/mL) and the bottle was filled to the mark either with H_2O or NaOH. Extracts were thoroughly mixed, divided into batches of 5 mL each, and kept frozen at −20 °C until needed. Further analysis was made according to a modified method proposed by Alén et al. (1985). In brief, the melted extract was passed through an ion-exchange resin column (Dowex 50 × 8–100, 8 mL), titrated with tetra-n-butylammoniumhydroxide (TBAH × 30, 0.02 M) until pH 9 and evaporated to dryness (35 °C) at reduced pressure. The tetra-n-butylammonia salts of acids were converted into their benzyl esters by adding reagent containing benzyl bromide in acetone (1:20). After that benzyl esters were analyzed using FID-GC (Agilent 6850). An external standard of a mixture of eight LWCA (formic, acetic, propionic, iso-butyric, butyric, isovaleric, valeric, and caproic) was used to obtain retention times of benzyl esters of each acid and the response factor for calculation of acid concentrations. Concentrations of acids are expressed as mg acid per kg of dry compost.

2.3. Phytotoxicity tests

The toxicity of composts to plants was tested with two methods – germination assay and plant growth bioassay. Germination assay is a quick and widely used method for evaluating compost phytotoxicity. However, it allows a rather short period of time to follow plant development. On the contrary, plant growth bioassay lasts longer, but it gives a better picture of the effect of compost on plant.

Germination assays were conducted on compost-water extracts (1:1, v/v). The extracts were prepared by mixing 300 mL of compost with 300 mL de-ionized water, shaken for 1 h at 180 rpm, centrifuged at 5000 rpm for 15 min, filtered through pre-rinsed filter paper (Whatman no. 4, Ø15 cm), divided into batches of 40 ml and frozen at −20 °C until needed. Five millilitres of extract, defrozen at 4 °C and equilibrated at room temperature, were added to a Petri dish (Ø9 cm) lined with filter paper (Whatman no. 1, Ø7.5 cm); de-ionized water was used as control. Twenty seeds of garden cress (*Lepidium sativum* L.) were placed in each Petri dish, closed with a lid, and incubated in the darkness at 25–27 °C. After 48 h, germination and length of radicle (accuracy 1 mm) were measured. The seed was considered to be germinated when the radicle was at least 1 mm long. Each plate had three replicates and an extract from each compost sample was tested five times. The experiment was accepted as valid when germination in control was above 95%. The compost was considered to be non-toxic, when the results of *t*-test ($p < 0.05$) showed no statistically significant difference between the parameter (germination or seedling length) in compost extract and in the control.

Plant growth bioassays were conducted on mixtures of compost and a peat-based growing medium (PBGM) at a 1:1 (v/v) ratio. PBGM (Finnpeat SI400, Kekkilä, Finland) was used as a control. Plastic pots (vol. 400 ml) were filled with either PBGM-compost mixture or PBGM and 50 seeds of garden cress (*L. sativum* L.) were spread on the surface and covered with a small amount of PBGM. The pots were placed in a greenhouse at a temperature of 25–27 °C, humidity 60% and 16/8 h day/night cycle. The pots were irrigated with de-ionized water on demand. The number of germinated seeds that appeared above the substrate was counted after 72 h of incubation and on the day of test termination. The test lasted 14 days. The seedlings were cut close to the substrate surface, dried at 70 °C and weighed (accuracy 0.01 g). The compost was considered to be non-toxic, when the results of the *t*-test ($p < 0.05$) showed no statistically significant difference between the parameter of the compost and the control.

2.4. Statistical analysis

All statistical analysis was performed with MS Office Excel 2003. The statistical significance of phytotoxicity was tested with *t*-test ($p < 0.05$). The correlation between phytotoxicity results and the other measured parameters (concentrations of LWCA, water-soluble NH_4–N and pH) is presented as bivariate square Pearson's correlation coefficients, and uses the time-series data for both.

3. Results and discussion

3.1. Temperature and gas emissions

The dynamics of temperature during the trial were typical for biowaste compost (Fig. 1). Although the biowaste was frozen at the beginning of the experiment, temperature reached its maximum values of about 60 °C in all three composters during the first week. The thermophilic stage lasted for about two weeks in control and in compost with additive B, and three weeks in compost with additive A. The results confirmed the patent statement for additive A (European Patent Office, 2004) that it prolongs the active phase of biowaste composting. However, Korhonen (2006) did not observe such an effect. Wong and Fang (2000) showed that the addition of lime to sewage sludge prolonged the thermophilic

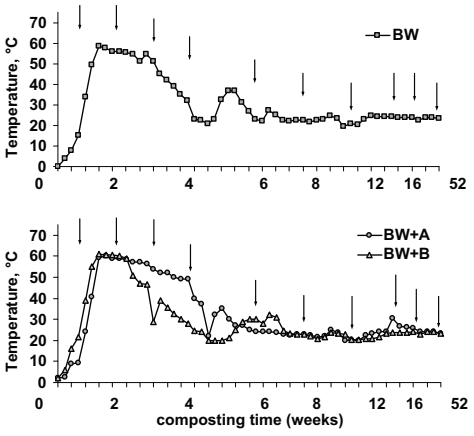

Fig. 1. Temperature dynamics during composting experiment. BW – biowaste compost, BW + A and BW + B – biowaste with additive A or B, respectively. Arrows indicate mixing points of compost.

stage and Koivula et al. (2004) reported 20 °C higher temperatures in biowaste compost with added bottom ash. However, no positive effect of additive B on temperature dynamics was observed in this trial, although the additive is of similar alkaline nature as the bottom ash or lime.

Because of a lack of gas flow data, gas concentration data are used to evaluate relative rather than absolute mass fluxes; still, the gas concentration data does help to provide a clearer overall interpretation of the results. The highest peaks of CO_2 were measured during the first week; maximum concentrations were 24.6 vol%, 16.4 vol%, and 23.6 vol%, in control, additive A and B containing composts, respectively. Slightly smaller peaks up to 15% were measured after five weeks of composting in control and compost with additive B. Concentrations <1% were reached by week 5 in BW + A compost and by week 7 in BW and BW + B composts. Low concentrations of ammonia gas (0.5–4 ppm) were registered after mixing the composter with additive B until week 6, but no emissions were measured in the control or the compost with additive A. Emissions of methane were below a detectable limit in all three composts.

3.2. pH and conductivity

The dynamics of pH in control and compost with additive A were typical for biowaste composting with peat as a bulking agent (Fig. 2). In these two composts a considerable rise in pH (from about 4.5 to 6) happened on the second week of composting. After that, pH fluctuated between 6 and 7 until the end of the experiment. A slight drop to 5.5 was noticed in the compost with additive A at the end of the test. Similar results were obtained by Korhonen (2006). In this trial, the addition of additive A did not have an immediate impact on pH when compared to a control, even though, from the patent specification (European Patent Office, 2004) the additive should raise pH after a week by 2–3 units. All experiments gave lower pH than often seen with biowaste composting because of the use of peat, which tends to have acidic properties.

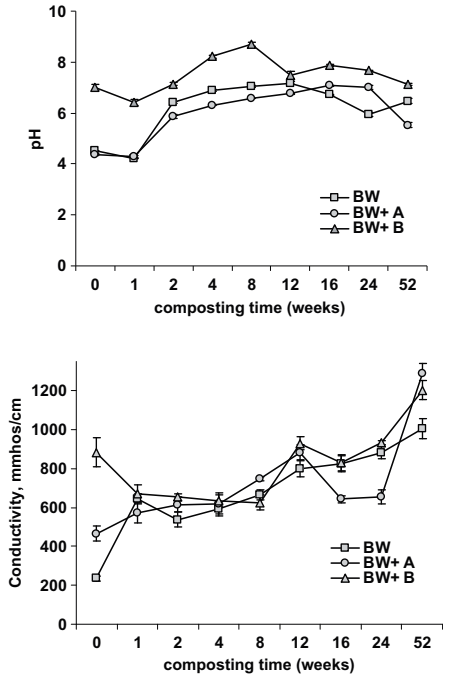

Fig. 2. Dynamics of pH and conductivity during composting process. BW – biowaste, BW + A and BW + B – compost with additive A or B, respectively. Error bars are standard deviations of the means of triplicates.

The addition of additive B had a significant impact on the pH of the compost, leading to a rise of initial values up to 7, and by week 8 it reached a maximum of 8.7, dropping gradually to 7.1 by the end of the trial. Such an impact was due to the composition of the additive, which mainly consisted of a strong alkaline compound, $Ca(OH)_2$. Alkaline pH was the reason for the volatilization of gaseous ammonia as it favours the formation of gaseous ammonia in comparison to the water-soluble form. A slight decrease in pH by the end of the experiment could be explained by the conversion of $Ca(OH)_2$ into $CaCO_3$ as a result of contact with water and carbon dioxide with further escape of hydroxide ion from compost with reject water (Adriano et al., 1980). Similar dynamics of pH were observed by Wong and Fang (2000) who tested the effect of lime on sewage sludge composting; during 100 days of composting pH dropped from 9.2 to 7.2. Fang et al. (1999) reported a similar change for coal fly ash added to sewage sludge.

As for electrical conductivity (EC), the addition of both compost additives significantly raised the initial values of this parameter (0.236, 0.465, and 0.882 mmhos/cm in the control, compost with additive A and B, respectively), but already after one week the difference between any three composts was not significant; the values were growing gradually as the process proceeded (Fig. 2). High conductivity of compost might cause toxicity to plants as high concentrations of salts decrease osmotic pressure between plant roots and growth substrate and thus affect water availability to the plant (Bewley and Black, 1994). According to ASCP Guidelines (2001) compost can be applied safely for plant growth if conductivity stays <2.5 mmhos/cm. Although EC values doubled during the experiment, reaching values of about 1.2 mmhos/cm, conductivity stayed in a range of acceptable limits and should not cause toxicity to plants.

3.3. Dry matter, ash content, and mineralization rate

The dry matter of the composts was about 40% at the beginning of the trial. During the first six months it increased to 45% in all three composts; during the next six months it continued to grow reaching 55% in BW, 64% in BW + A and 48% in BW + B by the end of week 52. Due to the high water holding capacity of peat, no free leachate was collected. Due to the mineralization of organic matter the relative amount of ash increased by 19%, 18%, and 17% in control, compost with additive A and B, respectively, which corresponds to 84%, 78%, and 71% degraded organic matter (Fig. 3). This means that the composting process was very successful in terms of the mineralization of organic matter. It is important to notice that almost 50% of degradation happened during the first 16 weeks. Looking at the dynamics of the process, it can also be noticed that during this time the mineralization rate in the compost with additive A was slower than in the other two composts. However, by the end of the experiment this difference was eliminated.

3.4. Nitrogen

The amount of total nitrogen (Table 2) increased about 1.5 times during the first twelve weeks of the test. During the following twelve weeks change was not so significant. The

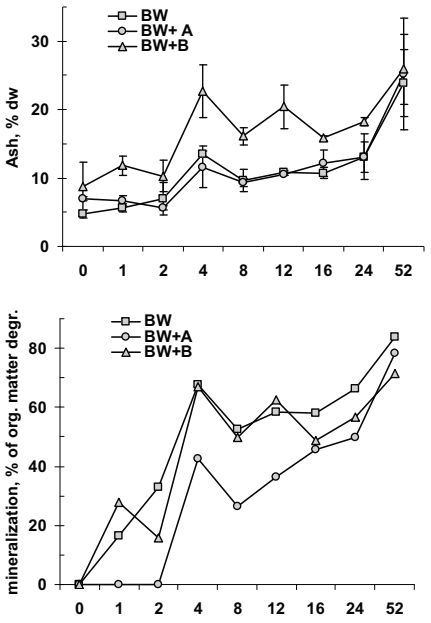

Fig. 3. Dynamics of ash and mineralization during composting experiment. BW – biowaste (control), BW + A and BW + B – compost with additive A or B, respectively. Error bars are standard deviation of the means measured in triplicates.

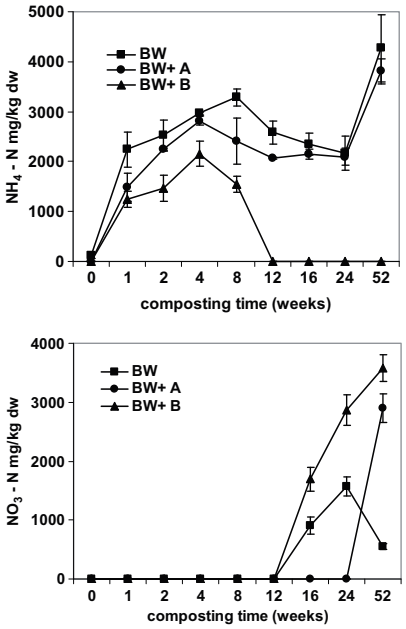

Fig. 4. Dynamics of water-soluble ammonium and nitrate during composting of biowaste (BW) and biowaste with additive A (BW + A) or B (BW + B); mean ± SD, measured in triplicates.

Table 2
Concentration of carbon and nitrogen analyzed with CN analyzer after combustion, and the C/N ratio in compost of different maturity level. BW – biowaste, BW + A and BW + B – biowaste with additive A or B, respectively.

	C (% dw)	N (% dw)	C/N ratio (% dw)
BW			
Start	48.9	1.55	32
12 wk	46.4	2.62	18
24 wk	47.7	2.77	17
BW + A			
Start	47.9	1.80	27
12 wk	46.5	2.60	18
24 wk	45.8	2.57	18
BW + B			
Start	46.2	1.65	28
12 wk	44.0	2.39	18
24 wk	44.6	2.43	18

difference could be explained by a higher degradation rate of organic matter during the first period, in comparison to the following twelve weeks.

Ammonification was rather strong in all three composts in the beginning of the experiment. The amount of water-soluble NH_4–N in control and compost with additive A grew continuously during the first eight weeks from almost zero to about 2500–3000 mg/kg dw. This was followed by a plateau during the next sixteen weeks and an increase to 4200 mg/kg dw in control and 3800 mg/kg dw in compost with additive A by the end of the experiment (Fig. 4). According to the patent of additive A (European Patent Office, 2004) it should reduce the concentration of NH_4–N after two months of composting, but this was not observed in this trial.

In compost with additive B, the dynamics were similar to other composts in the beginning, i.e. the amount of NH_4–N increased from almost zero to about 2000 mg/kg dw during the first four weeks, but dropped to below detectable limit by week 12 and remained at almost zero until the end of the trial. The decrease in NH_4–N was partly caused by volatilization of gaseous ammonia, due to the slight alkaline pH of the compost, which was recorded in its gas phase. However, the major contribution to the removal of NH_4–N appears to have been nitrification. The results of nitrification were recorded by week 16 as an increase in the concentration of water-soluble NO_3–N from almost zero to 1700 mg/kg dw, growing constantly to reach a maximum of 3500 mg/kg dw by the end of the experiment (Fig. 4). Possibly, slightly alkaline pH and dissolved oxygen, released as a result of CaO_2 degradation of additive B, favoured the high activity of nitrifying bacteria, which resulted in formation of NO_3–N (Sinha and Annchhatre, 2007). An increase in concentration of NO_3–N on week 16 was also recorded in the control; however, it was smaller (900 mg/kg dw) and further growth was not constant as in compost with additive B. In compost with additive A, a rise in NO_3–N (from almost zero to 2900 mg/kg dw) was registered only on week 52. Overall, from the dynamics of water-soluble NH_4–N and NO_3–N it can be concluded that the nitrification process was more effective in compost with additive B, which led to an earlier decrease in NH_4–N as well as an earlier increase in NO_3–N. Similar dynamics of NH_4–N as in compost with additive B were observed by Fang et al. (1999) with coal fly ash added to sewage sludge: the highest concentrations of ash (35%) caused the highest increase in NH_4–N and elevated loss by

volatilization. However, addition of any level of ash (10%, 25%, and 35%) did not have a positive effect on the formation of NO_3–N. According to the Decree on fertilizers issued by the Ministry of Agriculture and Forestry of Finland (656/01/2007, Appendix 1), if compost is used as a soil improver, the NO_3–N/NH_4–N ratio should be above 1. After one year of composting, only compost with additive B satisfied this condition; in BW compost the ratio was 0.13 and in BW + A 0.76.

3.5. Dynamics of low-weight carboxylic acids

In all composts concentrations of acids, both water- and NaOH-extractable, were the highest at the start of the trial (Table 3). The total amounts of acids extracted with NaOH were 4000, 2850, and 11,500 mg/kg dw in control, compost with additive A and B, respectively. Total concentrations of water-soluble acids were 1000, 470, and 10,600 mg/kg dw in control, compost with additives A and B, respectively. Amounts decreased rapidly by the fourth week of composting and after that continued to decrease slowly. After 52 weeks of composting, the total concentration of NaOH-extractable acids in composts was about 1000–1200 mg/kg dw and water-soluble 100–250 mg/kg dw. A rapid decrease of low-weight carboxylic acids at the beginning of composting is typical for the process (De Vleeschauwer et al., 1982; Manios et al., 1987) because the acids are utilized as an energy source and construction material in microbial activity (Madigan et al., 2003).

In general, only 10–40% of the LWCA was water extractable. However, acetic acid in the compost with additive B was almost 100% water extractable during the first two weeks of composting. Similarly, Baziramakenga and Simard (1998) reported that on average 34% of the total amount of acids extracted with NaOH from manure compost was water extractable.

The dominant acid in the compost with additive B was acetic, its concentration being about 10 times higher than in the two other experimental composts, while concentrations of formic, propionic and butyric acids were on the same levels as in the two other composts. Concentrations of iso-butyric, iso-valeric, valeric, and caproic acids were close to or below detectable limits (50 mg/kg dw) in composts of all ages, therefore they are not presented in Table 3. The dynamics of LWCA with molecular size of C_2–C_5 has been documented rather well (De Vleeschauwer et al., 1982; Ozores-Hampton et al., 1999); however, formic acid has been studied rather poorly. The reason could be that most methods used for analysis of LWCA are not suitable for determination of formic acid due to its small molecular size and structure. However, formic acid, as other LWCA, is an important intermediate product of microbial organic matter degradation. Data obtained in this work showed that the concentration of formic acid can be as high as acetic, as demonstrated in control and compost with additive A.

Accumulation of LWCA in compost is usually considered to be an indicator of anaerobic conditions (Epstein, 1997). However, more precisely, high concentrations of LWCA in the beginning of composting indicate a net surplus of the acids in a biodegradation – biosynthesis processes, but not necessarily oxygen availability. Wang et al. (2002) presented data where during degradation of kitchen garbage concentrations of formic, acetic, and succinic acids were several times higher in aerobic conditions compared to anaerobic. Although most of the studies have been done on LWCA formation by strict anaerobic and facultative anaerobic bacteria (Schlegel, 1988; Mata-Alvarez, 2003), there is also evidence that LWCA and, especially acetic acid, can be formed under aerobic conditions, too. For example, according to Andersen and von Meyenburg (1980) *Escherichia coli* excretes acetate as a major by-product of its aerobic metabolism. Acetate is produced from acetyl coenzyme A (CoA) via acetyl phosphate by the Pta–AckA (Pta, phosphotransacetylase; AckA, acetate kinase) pathway, which is reversible and constitutively expressed (Chang et al., 1999). Such mechanism might be common also for other microorganisms, because acetate is one of the most widely spread intermediate products of organic matter degradation (Lehninger et al., 1993). Data obtained in our study might also speak in support of aerobic formation of acetic acid.

The addition of additive B led to a quick release of oxygen from degradation of calcium peroxide, which had a boosting effect on microbial activity and a high production of acetic acid. A triggering effect of hydrogen peroxide on composting process was presented by Balis et al. (2002). The addition of additive A did not have such obvious significance on LWCA dynamics.

3.6. Macro- and micronutrients

Compared to the control, the compost with additive A had higher initial concentrations of Fe, Mn, Na, and S, while compost with additive B had elevated amounts of Ca, Cu, and Na (Table 4). High initial concentrations of Ca, Fe, Mn, and S can be explained by the nature of the additives as these elements are constituents of the additives' formula; elevated concentrations of other elements can be due to heterogeneity of stock material used for composting. Concentrations of all elements increased significantly during the first 12 weeks in all composts. During this time mineralization of organic matter was most active and as a result inorganic compounds became concentrated. An increase in mineral concentrations between weeks 12 and 24 was not as high as before.

3.7. Phytotoxicity

Germination and plant growth bioassays showed phytotoxicity of immature compost with slight difference in duration of phytotoxic period. In both tests seed germination of *L. sativum* was not affected by compost immaturity. Germination in compost was close to germination in the control. Seedling length and shoot weight were good parameters to reflect phytotoxicity (Fig. 5). Germination tests showed no consistent statistically significant difference between radical length in the control and extract of compost BW + B after 8 weeks of composting, and between composts BW and BW + A – after 24 weeks. In plant growth bioassay toxicity of compost BW + B was eliminated after 8 weeks, while after 16 weeks in BW and BW + A. So, both tests indicated that additive B shortened the toxicity period of immature compost at least by half, while additive A did not have a big impact on the parameter. Depending on the test method, toxicity of BW and BW + A lasted either for 24 (test duration 2 days) or 16 weeks (test duration 14 days). Possibly, immaturity of compost has a stronger effect on early seedling development which could be further eliminated as the plant grows.

Although phytotoxicity of immature compost is a well presented phenomena (Brinton and Tränkner, 1999; De Vleeschauwer et al., 1982; Ozores-Hampton et al., 1999; Tiquia and Tam, 1998; Wong and Chu, 1985; Zucconi et al., 1981), its detailed mechanism of action, duration, etc., are rather poorly understood, which can be explained by the complexity of the process due to the heterogeneity of stock material, differences in composting technology and management of the process. However, understanding of the phenomena is very important for the assessment of compost quality, especially if the material is used as soil improver. In our experiment, depending on the phytotoxicity test and presence/absence of improvers, the duration of compost toxicity lasted 8–24 weeks. The period of toxicity is similar to findings obtained by Zucconi et al. (1981), Wong and Chu (1985), and Tiquia and Tam (1998). In the research of Hartz and Giannini (1998), at least 9–12 weeks were required to minimize the negative impact of immature municipal yard compost. In an experiment conducted by

Table 3
Average concentrations (mg/kg dw) of formic, acetic, propionic, and butyric acids and total amount of acids (iso-butyric, iso-valeric, valeric, and caproic included) extracted with H_2O or NaOH from compost samples of different age. Compost samples are biowaste (BW), biowaste with additive A (BW + A) and B (BW + B). SD – standard deviation of four replicates.

	H_2O					NaOH				
	Formic	Acetic	Propionic	Butyric	Total	Formic	Acetic	Propionic	Butyric	Total
BW										
Start	382	333	160	135	1010	2398	908	137	398	3965
SD	149	70	40	37	296	275	6	10	151	462
1 wk	165	138	122	0	424	1097	1528	151	71	2870
SD	7	22	43	0	72	152	223	59	26	487
2 wk	76	102	55	20	253	791	1078	150	66	2098
SD	13	14	42	10	79	335	44	60	10	464
4 wk	197	153	164	0	534	427	750	186	65	1438
SD	149	78	59	0	308	112	147	82	21	380
8 wk	103	166	93	27	389	354	586	129	54	1123
SD	36	56	9	7	108	22	38	35	1	96
12 wk	137	168	58	17	380	428	741	278	70	1537
SD	58	117	73	20	267	32	53	40	6	155
16 wk	102	69	124	13	337	532	593	137	26	1308
SD	42	19	53	15	132	205	65	19	31	342
24 wk	98	69	345	0	512	435	600	331	73	1439
SD	9	16	169	0	194	25	44	181	47	297
52 wk	75	49	144	0	268	339	472	186	40	1037
SD	28	10	53	0	90	61	135	72	48	316
BW + A										
Start	178	231	28	17	472	1312	1051	109	265	11,549
SD	75	72	3	8	179	203	49	16	238	931
1 wk	296	182	129	43	669	1119	997	102	107	3479
SD	141	53	51	10	280	487	25	23	97	649
2 wk	239	147	87	0	473	550	1178	184	61	2967
SD	152	94	78	0	324	231	555	53	38	757
4 wk	108	95	27	8	257	485	808	119	13	1528
SD	34	30	8	5	99	95	109	12	2	470
8 wk	111	110	25	0	246	457	774	189	12	1196
SD	30	22	10	0	63	99	141	38	8	220
12 wk	109	97	29	0	261	492	717	372	10	1516
SD	27	48	12	0	118	78	121	46	11	212
16 wk	114	93	33	0	240	523	783	184	10	1100
SD	40	62	6	0	109	159	248	79	12	496
24 wk	88	56	0	0	145	518	646	196	13	1352
SD	7	20	0	0	27	89	113	109	9	235
52 wk	53	40	0	0	93	354	390	362	8	1226
SD	23	12	0	0	35	62	98	190	9	258
BW + B										
Start	386	9954	0	142	10,636	1186	9610	86	533	2844
SD	211	679	0	93	1048	127	560	75	131	554
1 wk	152	2041	121	63	2405	1340	1635	157	194	2416
SD	16	172	38	3	261	390	156	18	36	697
2 wk	96	1826	156	45	2210	686	1824	192	122	2090
SD	5	155	51	5	247	72	531	23	29	1055
4 wk	123	278	193	20	629	441	795	209	66	1425
SD	86	124	64	8	299	140	231	56	19	218
8 wk	68	55	60	0	183	362	622	170	43	1432
SD	21	16	69	0	106	66	129	20	5	286
12 wk	110	153	68	18	378	445	769	275	26	1591
SD	33	127	87	20	303	63	34	85	30	255
16 wk	91	31	334	12	480	351	563	157	30	1521
SD	11	42	143	14	224	120	224	110	42	523
24 wk	64	36	0	0	100	358	626	345	23	1374
SD	7	12	0	0	20	18	103	87	27	320
52 wk	44	30	0	0	74	325	629	187	85	1115
SD	23	4	0	0	27	86	45	92	35	360

De Vleeschauwer et al. (1982) compost from town refuse did not lose its toxicity before 17 weeks of composting.

Possible reasons for the toxicity of immature compost suggested in the literature are high concentrations of low-weight organic acids (Brinton and Tränkner, 1999; De Vleeschauer et al., 1982; Lynch, 1980; Shiralipour and McConnel, 1997), high concentrations of NH_4–N (Britto and Kronzucker, 2002; Tiquia and Tam, 1998), oxygen depletion (Brinton and Evans, 2002), or the presence of heavy metals (Epstein, 1997). Correlation coefficients of phytotoxicity results obtained in this experiment to various parameters are presented in Table 5. In general, coefficients of acids are higher than NH_4–N and pH. Surprisingly, correlation is higher for acids extracted with NaOH than with water, whereas the opposite could be expected. Probably, extraction with NaOH allows the assessment of potentially phytotoxic concentrations of acids in a better way than extraction with water, as acids slightly attached to compost matrix can become easily water-soluble in a dynamic situation of plant development. Due to lack of data the statistical significance of the correlation could not be proven valid; therefore more data is needed to support the above assumption.

Table 4
Concentrations of macro- and micronutrients (mg/kg dw) in composts after digestion with nitric acid in microwave oven. BW – biowaste compost, BW + A and BW + B – composts with additive A or B, respectively.

	B	Ca	Co	Cu	Fe	K	Mg	Mn	Na	P	S
BW											
Start	<5	11,500	<0.5	5	900	3800	1050	19	1250	900	1400
12 wk	7	12,000	1.0	18	2200	6000	1700	72	3500	2500	2200
24 wk	6	14,500	0.7	22	1500	6500	1400	61	3700	3200	2400
BW + A											
Start	<5	6500	0.8	8	2100	4000	1200	310	2200	1250	2500
12 wk	5	12,000	1.1	23	2400	6500	1700	310	3500	2800	3300
24 wk	6	11,500	1.1	20	3000	6000	1900	290	3200	1800	2900
BW + B											
Start	<5	29,000	<0.5	33	1150	3400	1300	31	2100	1100	1500
12 wk	6	40,000	0.7	14	1700	5600	1700	60	2800	1700	2100
24 wk	5	43,000	0.6	12	1400	5600	1700	54	2900	2200	2100

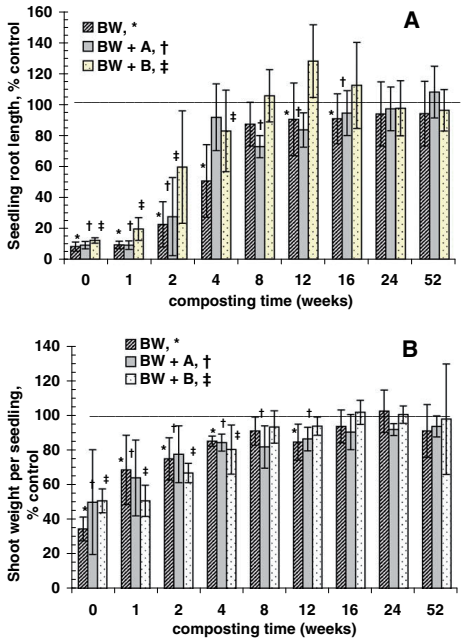

Fig. 5. Effect of composting process with and without additives on phytotoxicity of *L. sativum*. BW – biowaste compost, BW + A and BW + B – compost with additive A or B, respectively. Results of germination bioassay (A) are expressed as ratio of root length of seedlings in compost extract and control (de-ionized water); mean ± SD, five repetitions in triplicates. Results of plant growth bioassay (B) are expressed as ratio of shoot weight in compost and control (peat-based growing media); mean ± SD, three repetitions in duplicates. Black line at 100% indicates performance of the parameter in control. Symbols (∗, †, ‡) indicate statistically significant difference ($p < 0.05$) between the control and the compost.

The possibility of compost toxicity due to heavy metals is low. Concentrations stayed close or below detectable limits for As (<10 mg/kg dw), Cd (<0.2 mg/kg dw), Ni (<4 mg/kg dw), and Pb (<15 mg/kg dw). Detectable amounts of zinc and chromium were found in all three composts. Both compounds became concentrated due to mineralization of organic matter. During 24 weeks of composting, the concentration of chromium (mg/kg dw) in BW compost increased from 3.5 to 14, in BW + A stayed around 8, and in BW + B increased from 2.5 to 4; the concentration of zinc increased in BW compost from 13 to 83, in BW + A from 18 to 68, and in BW + B from 40 to 47. However, heavy metals become less mobile as composting proceeds (Greenway and Song, 2002; Walter et al., 2006) and therefore less available to plants.

Table 5
Square Pearson's correlation coefficients of two phytotoxicity measures to concentrations of acids (formic, acetic, and total) extracted with H_2O or NaOH, NH_4–N, and pH through time. Tested species – *L. sativum*. Substrates tested in germination bioassays were water extracts (A) or mixture with peat-based growing medium (B) of biowaste compost (BW), compost with improver A (BW + A) or improver B (BW + B).

	Formic		Acetic		Total		NH_4– N	pH
	H_2O	NaOH	H_2O	NaOH	H_2O	NaOH		
(A) Root length per seedling								
BW	0.47	0.57	0.31	0.71	0.22	0.74	0.35	0.48
BW + A	0.82	0.86	0.90	0.74	0.89	0.91	0.56	0.51
BW + B	0.53	0.78	0.60	0.51	0.60	0.60	0.04	0.39
(B) Dry weight per seedling								
BW	0.74	0.91	0.72	0.29	0.54	0.89	0.56	0.28
BW + A	0.49	0.71	0.49	0.53	0.67	0.92	0.72	0.09
BW + B	0.54	0.74	0.55	0.46	0.56	0.55	0.10	0.07

4. Conclusion

In summary, addition of additive with a high content of calcium hydroxide and the presence of calcium peroxide (improver B) had a significantly positive effect on the process and quality of compost. Its addition led to higher pH, faster elimination of water-soluble NH_4–N and increase in NO_3–N, and two times faster elimination of phytotoxicity. This conclusion could be sensitive to the use of peat as a bulking agent in these experiments, which led to lower pH and so additional importance for high alkalinity additives. A negative aspect was volatilization of ammonia. A mixture of mineral salts with clay (improver A) did not have so obvious positive results except for a one week longer thermophilic phase. Generally said, most of the positive aspects of the additive claimed in the patent were not confirmed in this study. An important finding of the study was that during composting concentrations of formic acid can be as high as acetic.

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III

A METHOD FOR MEASURING LOW-WEIGHT CARBOXYLIC ACIDS FROM BIOSOLID COMPOST

by

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A Method for Measuring Low-Weight Carboxylic Acids from Biosolid Compost

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ABSTRACT

Concentration of low-weight carboxylic acids (LWCA) is one of the important parameters that should be taken into consideration when compost is applied as soil improver for plant cultivation, because high amounts of LWCA can be toxic to plants. The present work describes a method for analysis of LWCA in compost as a useful tool for monitoring compost quality and safety. The method was tested on compost samples of two different ages: 3 (immature) and 6 (mature) months old. Acids from compost samples were extracted at high pH, filtered, and freeze-dried. The dried sodium salts were derivatized with a sulfuric acid–methanol mixture and concentrations of 11 low-weight fatty acids (C_1–C_{10}) were analyzed using headspace gas chromatography. The material was analyzed with two analytical techniques: the external calibration method (tested on 11 LWCA) and the standard addition method (tested only on formic, acetic, propionic, butyric, and *iso*-butyric acids). The two techniques were compared for efficiency of acids quantification. The method allowed good separation and quantification of a wide range of individual acids with high sensitivity at low concentrations. Detection limit for propionic, butyric, caproic, caprylic, and capric acids was 1 mg kg^{-1} compost; for formic, acetic, valeric, enanthoic and pelargonic acids it was 5 mg kg^{-1} compost; and for *iso*-butyric acid it was 10 mg kg^{-1} compost. Recovery rates of LWCA were higher in 3-mo-old compost (57–99%) than in 6-mo-old compost (29–45%). In comparison with the external calibration technique the standard addition technique proved to be three to four times more precise for older compost and two times for younger compost. Disadvantages of the standard addition technique are that it is more time demanding and laborious.

Amount of low-weight carboxylic acids (LWCA) in compost mass is an important parameter for the composting process as these acids can have a toxic effect to plants (Epstein, 1997). Additionally, LWCA are contributors to odor nuisance during the composting process. Therefore, choosing a proper method for LWCA analysis is a significant task for monitoring composting processes and avoiding toxicity to plants when compost is applied for plant growth. The purpose of this paper is to attract attention of researchers to the importance of choosing an analytical method for analysis of LWCA in composts of different age.

In many published studies focusing on LWCA dynamics in compost, methods used for acids analysis are slightly modified from the methods applied to different matrixes, for example, wastewater (Brinton, 1998), waste material (Schuman and McCalla, 1976), and soil (Kirchmann and Widén, 1994; García et al., 1991). In these references and many others it is not mentioned whether suitability of the method for the compost matrix was tested. All the methods mentioned above are based on distillation of acids at low pH and their analysis using liquid or gas chromatography. This procedure leads to losses of volatile substances as in acidic conditions LWCA are in the most volatile form. One preferable option for acids extraction from compost is using solution with basic pH, which leads to formation of acid salts and prevents losses of substance due to volatilization. A number of articles contain examples of this type of extraction method (Elliott and Travis, 1975; Brinton, 1998; Baziramakenga and Simard, 1998). This method also allows storage of extracted salts for a rather long time before analysis is conducted, which makes it more convenient.

In all the references mentioned above LWCA were analyzed using so-called external calibration methods, when acids in the unknown sample are analyzed and concentration of compounds calculated on the basis of a calibration graph obtained from the analysis of standard solution. Although the external calibration method is widely applied, the differences in pH, ionic strength, temperature, impurities, and sample matrix structure, which are very typical for compost mass, may interfere or change the analytical signal produced by the chromatograph and cause erroneous results (Bader, 1980). The standard addition method overcomes this problem. In this case the acids are directly added to the sample, which increases the peak area in the chromatogram. By making a series of additions, the original unknown concentration of component can be calculated by extrapolating the straight line built from a series of points until it crosses the abscissa, which represents the absolute value of the original concentration (Robards et al., 1997), or in other words, the amount of compound both extracted and trapped in the matrix.

Another aspect is that methods differ in a range of acids that can be analyzed with them. In many articles variety of acids mentioned is rather small. For example, García et al. (1991) report data on acetic, propionic, *iso*-butyric, and butyric acids studied in water extracts of sewage sludge compost; Chanyasak et al. (1982) present results of two groups of compounds in water extracts of composted garbage (acetic and other low fatty acids); and DeVleeschauwer et al. (1982) discuss dynamics of acetic, propionic, *iso*-butyric, butyric, and *iso*-valeric acids in refuse compost. Very few articles contain information on dynamics of formic acid, which is an interesting compound due to the structure, small size, and activity of the acid molecule. Usually restrictions for a

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Abbreviations: LWCA, low-weight carboxylic acids.

variety of acids that can be analyzed are defined by analytical instruments and columns that are used in chromatographic analysis.

The method described in this work has been designed with the intention that special features of compost, like maturation, absorption of compounds to compost matrix, volatilization of LWCA, and microbial degradation, could be minimized. The method has been developed at the Technical Research Center of Finland and has been previously applied for other materials (e.g., bread and diary products). The objectives of the present research were to (i) measure the amounts of a wide range of low-weight fatty acids (C_1–C_{10}) in compost by applying basic extraction–derivatization and headspace sample injection, (ii) compare the amount of low-weight fatty acids (C_1–C_4) analyzed by the external calibration and standard addition methods, and (iii) calculate and compare recovery rates of small quantities of LWCA from compost samples of two different ages.

MATERIALS AND METHODS

Sampling and Preparation of Compost Material

Samples of biosolid compost were collected at the full-scale composting plant of the Helsinki Metropolitan Area Council, Finland. At the plant, source-separated biowaste mixed with wood chips is processed in composting tunnels for 2 wk and after that transferred outdoors and piled in windrows for maturation. Samples for experiments were collected from two piles of different age: 3 and 6 mo old. Compost of two different ages was chosen to study applicability of the method for analysis of mature and immature composts, which have different chemical and microbial structures. The samples were packed in polyethylene bags (200-L volume) and transported to the laboratory. Upon arrival samples were sieved through a 5-mm screen, thoroughly mixed, divided into approximately 1-L subsamples, and stored frozen at −20°C in the sealed plastic bags. Subsamples were used for LWCA analysis when needed.

Dry matter, ash content, pH, and conductivity of the samples were analyzed according to standard methods CEN 13039, CEN 13037, and CEN 13038, respectively (European Committee for Standardization, 1999a, 1999b, 1999c). Concentrations of dissolved ammonium, nitrate, and nitrite ions in compost water extract (1:5) were detected using test strips (Aquamerk for NH_4^+ and Merckoquant for NO_2^- and NO_3^-; Merck, Darmstadt, Germany). Phytotoxicity of compost samples was tested with a plant bioassay. The tested mixture was compost and peat-based growth medium (1:3). The control was peat-based growth medium; the tested plant was garden-cress pepperweed (*Lepidium sativum* L.), 100 seeds per pot, with four replicates. Plants were grown in a phytotron. The temperature was 25°C and relative humidity 60%, with a day–night regime of 16 and 8 h, respectively. Cultivation lasted for 8 d, after which germination percent and dry weight of seedling shoots were measured. Results are presented as percent germination or weight to control.

Analysis of Low-Weight Carboxylic Acids

Calculation of LWCA concentrations in compost mass and their recovery rates was done in two stages. First, amounts of LWCA (background levels) were analyzed and calculated using the external calibration method and then the standard addition method.

External Calibration Method

For the external calibration analysis, 5 g of subsample, unfrozen at 4°C, was weighted into a glass beaker (500-mL volume), 50 mL of 0.1 *M* NaOH was added to the sample, and the mixture was homogenized with a blender (Bamix, Mettlen, Switzerland) for 2 min (pH >12). The slurry was filtered through a prerinsed filter paper (Number 4, ∅ 15 cm; Whatman, Maidstone, UK) into a 100-mL glass volumetric flask. The beaker was rinsed twice with 15 mL of 0.1 *M* NaOH and washings were filtered through the same filter paper. After all the solvent was drained, the flask was filled to the mark with 0.1 *M* NaOH and shaken.

One- and ten-milliliter aliquots were pipetted into 22-mL headspace vials and the vials were covered with foil and frozen at −20°C. Each aliquot had two parallels. Frozen aliquot was dried in a vacuum drier and stored at room temperature covered with aluminum foil. Before analysis freeze-dried aliquot was derivatized with 2 mL of methylating reagent (163 mL 50% sulfuric acid + 138 mL methylalcohol), sealed immediately with a Teflon-faced rubber septa and aluminum seal, and temperated at 80°C in a water bath for 10 min. Methylated samples were cooled down to room temperature and used for LWCA chromatographic analysis.

The acid methyl esters were analyzed with a gas chromatograph (HP5890 Series II; Hewlett-Packard, Palo Alto, CA), which was interfaced to a static headspace autosampler (Tekmar 7000; Teledyne Tekmar, Mason, OH). The samples were equilibrated at 80°C for 30 min. Pressurization time was 0.5 min, pressure equilibrate time 0.25 min, loop fill time 0.15 min, and loop equilibrate time 0.05 min. Both sample loop and transfer line temperatures were 180°C. The gas chromatograph was equipped with a 60-m × 0.53-mm RTX-1701 column (SGE, Austin, TX) and the flame ionization detector operated at 250°C. The injector temperature was 220°C. The oven temperature program was the following: from −20 to 130°C temperature rise rate was 5°C min^{-1}, from 130 to 240°C rise rate was 20°C min^{-1}, and time at 240°C was 6.5 min. Helium was used as a carrier gas with a velocity rate of 25 cm s^{-1}. The split ratio was 1:30. Injection volume was 1 mL. Concentration of each acid was calculated based on the peak area of acids in the sample and standard solution of acids. Number of replicates analyzed by this technique was 14.

The following acids were identified with the described technique: formic (methanoic), acetic (ethanoic), propionic (propanoic), butyric (*n*-butanoic), *iso*-butyric (*iso*-butanoic), valeric (*n*-pentanoic), caproic (*n*-hexanoic), enanthic (*n*-heptanoic), caprylic (*n*-octanoic), pelargonic (*n*-nonanoic), and capric (*n*-decanoic).

Standard Addition Method

For conducting analysis of LWCA with the standard addition method five acids were added (formic, acetic, propionic, butyric, and *iso*-butyric) to each compost sample at increasing concentration in proportion to the background concentration measured with the external calibration method. The amounts of acids added are presented in Table 1.

A corresponding amount of each acid was added to a 5-g compost sample and weighted into a glass vial (38-mL volume). The vial was closed immediately with a screw lid, thoroughly shaken, and allowed to stand for 2 h at room temperature so that the compost could absorb the acids but the microbes existing in compost would decompose the acids as little as possible. Twenty milliliters of 0.1 *M* NaOH was added in to the vial. After mixing the compost mass was allowed to stand for another 30 min. Content of the vial was transferred

Table 1. The amount of individual low-weight carboxylic acids (LWCA) added to 3- and 6-mo-old compost samples for conducting the standard addition method of LWCA analysis.

	3-mo-old compost						6-mo-old compost					
Acid	B_3†	A_1‡	A_2	A_3	A_4	A_5	B_6†	C_1‡	C_2	C_3	C_4	C_5
	mg acid kg^{-1} compost											
Formic	591	1182	2364	3545	4727	5909	440	880	1760	2640	3520	4400
Acetic	1818	3636	7273	10909	14545	18182	560	1120	2240	3360	4480	5600
Propionic	6	12	25	38	50	63	10	20	40	60	80	100
Butyric	4	9	18	27	36	45	5	10	20	31	41	52
Iso-butyric	3	6	12	19	25	31	3	7	14	21	28	36

† B_3 and B_6 are the amounts of acid analyzed in the original 3- and 6-mo-old compost samples (background).
‡ A_n and C_n are the amounts of acid added to the same vial.

into a glass beaker (500-mL volume), the vial rinsed twice with 15 mL of 0.1 *M* NaOH, and washings combined to the same beaker. The sample was homogenized for 2 min and filtered through a prerinsed filter paper (Number 4, 15 cm; Whatman) into a 100-mL glass volumetric flask. After all solvent was drained, the bottle was filled to the mark with 0.1 *M* NaOH and shaken. For methylation and analysis procedures, see the External Calibration Method section (above). Each vial had four replicates.

Results of the standard addition method were used for recalculation of acid concentrations in 3- and 6-mo-old composts. For each acid it was done based on five concentration points. For a detailed equation refer to Harris (1999).

Recovery rate of each acid was calculated based on results of the standard addition method. Calculations were done according to the formula:

$$\text{recovery rate } (\%) = (A_{\text{sample}} - A_{\text{background}}/A_{\text{standard}}) \times 100\%$$

where A_{sample} is peak area of acid determined in the sample after addition of acids; $A_{\text{background}}$ is average peak area of acid in the original compost sample; and A_{standard} is peak area of acid determined in standard solution.

Statistical Analysis

Concentrations were analyzed by two different analytical methods. Significance of differences between the mean concentrations measured with two methods was tested with a *t* test using SPSS 12.01 (SPSS, 2003). To minimize effect of difference in variability and in number of replicates (14 for the external addition and 4 for the standard addition tests) concentrations to be compared were log transformed before statistical analysis. Differences in variability between the two treatments were tested with Levene's test using SPSS 12.01.

RESULTS AND DISCUSSION

Results of the basic analysis and plant bioassay of compost samples are presented in Table 2.

Application of Basic Pretreatment and Headspace Gas Chromatography for Low-Weight Carboxylic Acids Analysis

Extraction of LWCA from compost samples with basic solution and further derivatization with methanol at low pH was an effective pretreatment method that allowed successful identification and quantification of acids with headspace gas chromatography. In spite of a high number of "noise" peaks produced by compounds originated from compost and solvent, peaks of 11 methyl esters of low-weight fatty acids (from formic to capric) could be clearly identified using standard solution. Figure 1 exhibits an example of a chromatography profile of 3-mo-old compost sample to illustrate peak shape. Although peaks of some methyl esters are small due to low concentrations, they are sharp and narrow and good for integration. The pretreatment method allowed us to prevent evaporation of volatile substances and store treated samples for up to 2 wk before analysis of acids without affecting the final result. This increases convenience of method application especially when a large number of samples should be analyzed. Therefore, the method gives an opportunity to study dynamics of these acids during the composting process in the future and investigate the role of LWCA in generating noxious odors during the composting process as well as toxicity to plants. In this research we have concentrated only on 11 carboxylic acids with the smallest molecular size, but this method has potential for analysis of a broader range of carboxylic acids, although this might need some tuning of chromatograph parameters.

Although 1- and 10-mL aliquots were used for LWCA analysis, in further discussion results of 10-mL aliquots only will be used because amounts of acids in 1-mL aliquots were close to detection limits of the method. Detection limits of individual acids with both methods varied considerably. For propionic, butyric, caproic, caprylic, and capric acids it was 1 mg kg^{-1} compost; for formic, acetic, valeric, enanthoic, and pelargonic acids it was 5 mg kg^{-1} compost; and for *iso*-butyric acid it was 10 mg kg^{-1} compost.

Table 2. Basic characteristics of 3- and 6-mo-old composts and results of plant growth bioassay on gardencress pepperweed (*Lepidium sativum* L.).

Parameter	3-mo-old compost	6-mo-old compost
pH	7.23	8.93
Conductivity, mS cm^{-1}	2.03	2.27
Dry matter, %	61.6	53.4
Ash content, %	34.8	46.6
Dissolved NH_4^+, g kg^{-1} dry compost	2.42	1.28
Dissolved NO_2^-, g kg^{-1} dry compost	0	0.064
Dissolved NO_3^-, g kg^{-1} dry compost	0	0.322
Germination, % control	94.8 ± 4.24a†	98.2 ± 4.08
Dry weight/seedling, % control	76.7 ± 2.2ab	82.9 ± 3.44ab

† Results of the plant bioassay test were compared using *t* test: a = significant difference to control ($p < 0.05$), b = significant difference to another sample ($p < 0.05$).

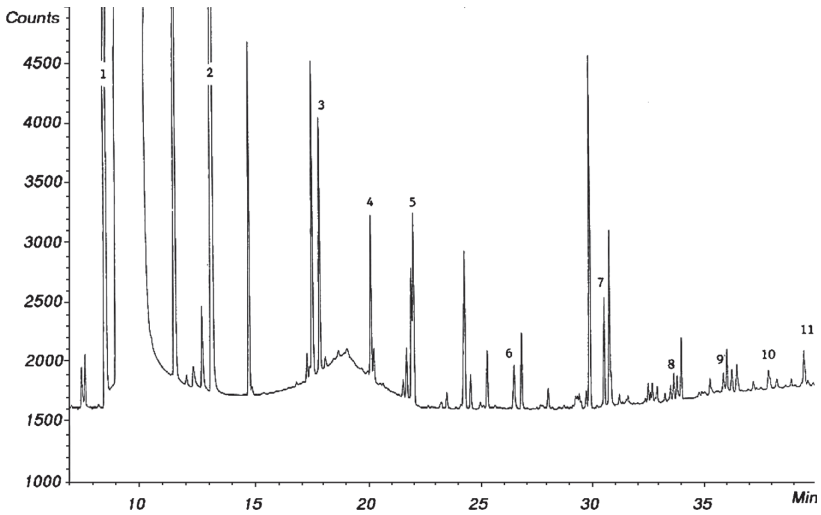

Fig. 1. Typical gas chromatography profile of 3-mo-old compost sample extracted at basic pH and derivatized with methanol at low pH. Peaks of methyl esters of formic (1), acetic (2), propionic (3), *iso*-butyric (4), butyric (5), valeric (6), caproic (7), enanthoic (8), caprylic (9), pelargonic (10), and capric (11) acids are sharp, symmetric, and quantifiable.

As it was described in Materials and Methods, the standard addition method was tested on five fatty acids only: formic, acetic, propionic, *iso*-butyric, and butyric. Linear response of standard solutions of these acids is presented in Fig. 2. Among all acids *iso*-butyric acid was the most sensitive and easy to identify, the most difficult to measure was acetic acid. Similar results were obtained by Paul and Beauchamp (1989) claiming that among three acids (acetic, propionic, butyric), acetic acid was the most difficult to measure especially at low concentrations.

Percent recovery of added fatty acids is presented in Table 3. In samples of 3-mo-old compost average recovery rates were higher than in 6-mo-old composts. For 3-mo-old samples the percentage was in a range of 57 to

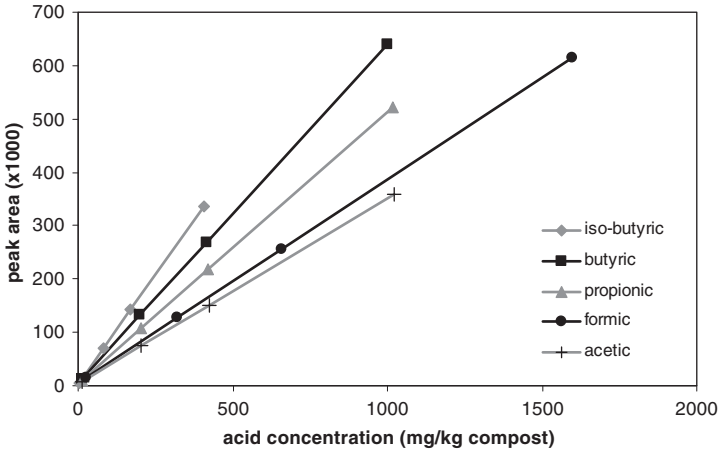

Fig. 2. Linear response of low-weight carboxylic acids (LWCA) standard solutions to increasing acid concentrations. The most sensitive and easiest for identification is *iso*-butyric acid, while the most difficult is acetic acid.

Table 3. Average recovery rates of low-weight carboxylic acids (LWCA) in 3- and 6-mo-old biowaste compost samples pretreated with a basic solution and analyzed with headspace gas chromatography.

Acid	3-mo-old compost	6-mo-old compost
	% (average ± SD)	
Formic	67.4 ± 3.6	45.0 ± 5.0
Acetic	71.0 ± 3.9	46.2 ± 5.6
Propionic	57.4 ± 5.8	35.0 ± 12.2
Butyric	99.8 ± 25.0	32.6 ± 9.1
Iso-butyric	62.4 ± 7.0	29.8 ± 4.0

99% and for 6-mo-old compost it was 29 to 45%. In older samples recovery rates of formic and acetic acids were higher than propionic, butyric, and *iso*-butyric acids; in younger samples no similar trend could be observed. These percentages were lower than reported by Paul and Beauchamp (1989), who tested the volatile fatty acid standard addition method on silt loam soil. They obtained recovery rates of acetic, propionic, and butyric acids ranging from 94 to 114% in a 3:1 water to soil extract. Lower recovery rate in this study especially in older sample can be explained by the structure of compost matrix. As compost is getting more mature and amount of complex organic molecules (e.g., humic substances) increases, they bind LWCA more tightly and make compounds more difficult for extraction. Therefore, the matrix effect plays a very significant role in analysis of LWCA. This assumption may bring an important aspect in selecting the proper analytical method when studying dynamics of LWCA during maturation of compost.

Standard Addition versus External Calibration

Concentrations of LWCA in compost samples were analyzed using both the external calibration and standard addition methods. Comparison of acid concentrations analyzed by the two methods is presented in Fig. 3 and 4. Results of both methods showed that formic

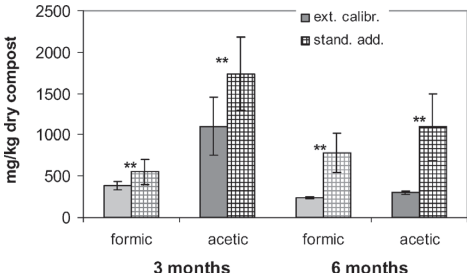

Fig. 3. Concentrations of formic and acetic acids analyzed by the external calibration and standard addition methods in 3- and 6-mo-old compost samples (mean ± SD; for external calibration $n = 14$, for standard addition $n = 4$). Amounts of acids analyzed by the standard addition method are significantly higher than by the external calibration method (indicates significance at the 0.01 probability level), especially in 6-mo-old compost, where the difference is three to four times.**

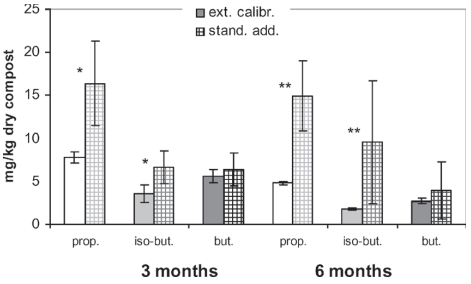

Fig. 4. Concentrations of propionic, butyric, and *iso*-butyric acids analyzed by the external calibration and standard addition methods in 3- and 6-mo-old compost samples (mean ± SD; for external calibration $n = 14$, for standard addition $n = 4$). Amounts of acids analyzed by the standard addition method are significantly higher than by the external calibration method (* indicates significance at the 0.05 probability level, ** significance at the 0.01 probability level). Only for butyric acid is the difference not significant.

and acetic acids were dominating in composts of both ages; their concentrations several hundreds times exceeded concentrations of propionic, butyric, and *iso*-butyric acids. Baziramakenga and Simard (1998) in their work have obtained similar data stating that both acetic and formic acids were dominating in composted manures. It is a very interesting fact that these acids were of similar concentration levels, because a lot of authors claim that on a large range of LWCA only acetic acid plays major role in producing phytotoxic effects (DeVleeschauwer et al., 1982; Lynch, 1977). Such a conclusion is made in those studies where concentration of formic acid was not measured. Results of a bioassay test (see Table 2) showed that compost of both ages significantly suppressed plant growth of cress salad. Therefore, it might be assumed that high concentrations of both formic and acetic acids could be the reason for it. But a toxicological study of acids is needed for proving this hypothesis.

Statistical analysis of results showed that the standard addition method had higher variability than the external calibration method, which could be due to the extrapolation procedure of several points of the sample. But even in spite of high variability the standard addition method allowed us to analyze significantly higher concentrations of all acids except butyric. An interesting fact is that for more mature compost the difference between means of the methods was three to four times, while for younger compost the difference was about two times. Again this proves the idea that choosing an improper analytical method can give a substantially erroneous result especially in analyzing LWCA in older composts.

An advantage of the standard addition technique is that it allows us to obtain more precise data on concentrations of LWCA in compost samples in comparison with the external calibration technique, as it takes into consideration the effect of acid entrapment to the compost matrix. Disadvantages of this technique are that it is laborious, time consuming, and costly.

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PHYTOTOXICITY OF LOW-WEIGHT CARBOXYLIC ACIDS

by

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Phytotoxicity of low-weight carboxylic acids

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ABSTRACT

Presence of low-weight carboxylic acids (LWCAs) can be the reason for phytotoxicity of green manures, treated bio-waste or digestates from biogas production applied to soils. As the phytotoxic concentrations of LWCA are poorly known, this work presents data on six acids (C_1—C_6: formic, acetic, propionic, butyric, valeric, and caproic). Phytotoxicity was measured in acute (72 or 120 h) and subchronic (21 d) assays for seed germination, seedling elongation, and plant growth for garden cress *Lepidium sativum* and ryegrass *Lolium multiflorum*. The dose–response relationship was modeled using Weibull model. Results showed a trend that toxicity of LWCA increases with the length of the carbon chain, formic acid (C_1) being the least and caproic acid (C_6) the most toxic. EC50 values in the acute seed germination of cress ranged between 1.9 and 4.2 mM and for ryegrass between 1.8 and 6.4 mM. In subchronic assays EC50 values for germination were in a range from 11 to 46 mM kg^{-1} dm for cress, and from 18 to 127 mM kg^{-1} dm for ryegrass. EC50 values for early seedling growth of cress based on acute assays ranged from 0.7 to 2.3 mM and that of ryegrass from 1.2 to 1.8 mM. Range of EC50 values for shoot biomass of cress was between 8 and 40 mM kg^{-1} dm and of ryegrass between 12 and 93 mM kg^{-1} dm.

1. Introduction

Low-weight carboxylic acids (LWCAs) are known as agriculturally important inhibitors of crops (Ulbright et al., 1982). Studies showed accumulation of LWCA in natural soils (Stevenson, 1967) and during application of plant materials or green manure followed by water logging (Chandrasekaran and Yoshida, 1973). LWCA are also found in treated biogenic materials like composts (Brinton and Tränkner, 1999; Himanen and Hänninen, 2011), digestates from biogas production (Paavola and Rintala, 2008) and stillages from bio-fuel production (Kaparaju et al., 2009) that are utilized or have potential to be utilized in agriculture or horticulture, and may exhibit phytotoxicity.

LWCA are essential metabolic by-products of microbial degradation of organic matter in aerobic, hypoxic and anoxic conditions (Schlegel, 1986). Among carboxylic acids with one to six carbons, formic acid (C1) is the smallest by its molecular weight and caproic (C6) the largest. Common for all, their carboxylic group dissociates in water solutions into carboxyl radical and hydronium ion. Degree of dissociation is dependent on pK_a of the acid and pH of the solution (Harris, 2003). LWCA are volatile or semi-volatile in nature. Henry's law constants range from 1320 to 5530 kg atm mol^{-1} at NTP conditions (Khan and Brimblecombe, 1992). So, in addition to phytotoxicity, pungent and rancid odor is usually an important nuisance in application of LWCA – containing substrates (Tolvanen et al., 1998).

Although phytotoxicity of LWCA is a known phenomenon, solid data on effective concentrations (EC values) is scarce. In numerical studies on dose–response relationships of LWCA conducted so far, effective concentrations were not calculated (Prill et al., 1949; Takijima, 1964; Rao and Mikkelsen, 1977; Ulbright et al., 1982; Manois et al., 1987). Therefore, utilization of the results in risk assessment of the treated biogenic materials is challenging. An important aspect is also that earlier experiments were conducted mostly on unbuffered acid solutions and lasted only long enough to follow effect on early stages of seed development. However, true growth media and soils are solid substrates, which have an impact on plant development for the whole lifetime. An important question is how data from the short tests in liquid solution may be used to predict the long-term endpoints, e.g. growth of plants in solid substrates? An attempt to study long-term effect of five LWCA in different types of soils was made by Chandrasekaran and Yoshida (1973) using a 23 d assay with rice seedlings. However, the assay was conducted with only two doses of the LWCA (5 and 10 mM kg^{-1}) and results of the test are difficult to extrapolate to other concentrations. In this research an attempt was made to obtain such results that can be utilized directly for evaluation of the phytotoxicity potential and risk assessment of the treated biogenic materials used for plant growth.

The aims of this study were: (1) to obtain data on acute and subchronic phytotoxicity of six LWCA for germination, early seedling

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development and growth using two species, monocotyledonous ryegrass and dicotyledonous cress, (2) to model dose–response relationships of the measured parameters, and (3) to evaluate toxicity potentials of LWCA based on EC values.

2. Materials and methods

2.1. Acute phytotoxicity assays

The aim of the acute assays was to obtain data on effects of LWCA on seed germination and early development of seedlings. The assays were conducted on a series of dilutions with seven concentrations. For formic, acetic, propionic, valeric and caproic acids the nominal concentrations were 0.1, 0.3, 0.6, 1.2, 2.4, 4.8, and 9.6 mM, and for butyric acid 0.1, 0.3, 0.61, 1.21, 2.42, 4.84, and 9.68 mM. Choices of the concentrations were based on the preliminary range – finding experiments. De-ionized water was used as control. For assays bottoms of Petri dishes (∅ 90 mm) were lined with filter paper (Whatman No.1, ∅ 70 mm) and moisturized with 10 mL of acid solution or control. Each plate was seeded with 20 seeds of garden cress *Lepidium sativum* (Habitec seed supplier, Finland) or ryegrass *Lolium multiflorum v. Fabio* (Tilasiemen seed supplier, Finland) and covered with the lid. Petri dishes were kept in incubator (Bio 1600, Weiss Umwelttechnick GmbH, Germany) in the dark at 25 ± 1 °C for 72 (garden cress) or 120 h (ryegrass). After the incubation, number of the germinated seeds was counted and total length of the seedlings (root + shoot) for garden cress or the longest root in each individual for ryegrass was measured with the accuracy of 1 mm. The seed was considered to be germinated when radicle was over 1 mm long. One experimental set consisted of one Petri dish for each concentration of LWCA and eight dishes for control, and was repeated seven times for cress and 12 times for ryegrass. An experiment was considered successful when the germination of cress in controls was over 95% and that of ryegrass 87%.

2.2. Subchronic phytotoxicity assay

The aim of the subchronic assays was to obtain data of LWCA effect on germination and plant growth. The assays were conducted according to ISO 11269-2 standard (ISO, 2008) with garden cress and ryegrass. Five dilutions were prepared for each acid (supplementary data, Table S1). The inorganic growth substrate was a mixture (6 + 1, v/v) of coarse-grained sand (particle size 0.5–1.2 mm; Maxit Oy, Finland) and the quartz sand (particle size 0.2–0.005 mm; NFQ Nilsiän kvartsi, SP Minerals Oy, Finland). Hundred milliliters of particular LWCA solution or control (de-ionized water) were added to 1.6 L of the growth substrate. To prepare one experimental unit, pre-rinsed plastic pot (∅ 100 mm, height 80 mm) was filled as following: approx. 1 cm of peat layer (Kekkilä Kasvuturve B2, Kekkilä Oy, Finland) on the bottom, 400 mL of the inorganic substrate, 25 seeds, and on top approx. 1 cm of the peat. Peat layers were used to prevent leaching of the substrate from the pot during watering and to decrease stripping of the acids from the substrate. The pots were held in the incubation chamber at 25 ± 4 °C with light/dark regime 16/8 h, the lightning being 13 000 l× with 185 μmol m^{-2} s^{-1} and color 6500 K (Biolux, Osram, Germany). The plants were watered with general fertilizing solution (NPK 12-6-9, Kekkilä Kukkaravinne, Kekkilä Oy, Finland) every or every second day to avoid drying of the substrate. Incubation time was 21 d, after which the plants were cut close to the substrate surface, dried at 70 °C overnight and weighted. Each experiment consisted of two replicates for each acid concentration and was repeated three times.

2.3. Measurement of pH

Before the start of the acute experiments pH was measured directly from the solutions (MeterLab PHM220, Radiometer Analytical, France). However, after the incubations pH was not measured due to lack of the solution volume. In subchronic experiments, pH

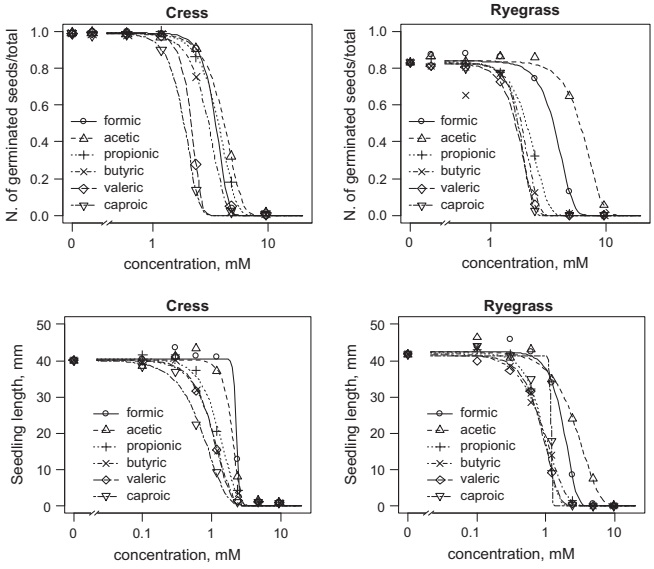

Fig. 1. Dose–response models for LWCA for 72 or 120 h germination and seedling growth of *L. sativum* (cress) and *L. multiflorum* (ryegrass) obtained in acute assay.

of the substrates was measured from a substrate–water solution (1 + 5, v/v) according to the standard CEN 13037 (CEN, 1999) before and after incubations. Measured pH values were used for calculations of the dissociated fractions of LWCA in the substrates using Henderson–Halsselbalch equation (Serjeant and Dempsey, 1979).

2.4. Modeling of dose–response relationships

Dose–response relationships, EC-values and their standard errors were calculated for each LWCA and endpoint (germination, early seedling growth and shoot biomass) using R program (version 2.10.1) drc-package. Weibull model 1.3 was used for data extrapolation. The model is applicable when the response pattern follows a non-linear curve, it is not symmetric around any point and lower limit is equal to zero. The model is described by equation:

$$f(x,(b,d,e)) = d\exp\{-\exp\{b(\log(x)-e)\}\}$$

The parameter d is the lower limit, b is the relative slope around e, and e is the logarithm of the inflection point (Ritz and Streibig, 2005).

3. Results

3.1. Dose–response relationships and EC values of LWCA

Dose–response relationships of LWCA for seed germination, early seedling growth, and plant growth were modeled using Weibull 1.3 multiple model (Figs. 1 and 2). The models were used to calculate effective concentration values such as EC10, EC50, and EC90 for each LWCA (Tables 1 and 2).

EC50 values of all LWCA obtained in acute assays for seed germination of cress fell in a range from 1.9 to 4.2 mM and for ryegrass from 1.8 to 6.4 mM. In subchronic assays EC50 values for delayed germination were in a range from 11.1 to 45.9 mM kg^{-1} dm for cress, and from 17.5 to 126.5 mM kg^{-1} dm for ryegrass (Table 1). Regarding to the early seedling growth EC50 values for cress were in a range from 0.7 to 2.3 mM and of ryegrass from 1.2 to 1.8 mM. Range of EC50 values for shoot biomass of cress were between 8.1 and 39.5 mM kg^{-1} dm and of ryegrass from 12.4 to 93.2 mM kg^{-1} dm (Table 2). Variability in acute assays was slightly lower than in subchronic assays. In general, relative standard error (RSE) varied from 2% to 10% in acute assays and from 5% to 12% in subchronic assays.

Considering EC10–EC90 intervals, transition from non-toxic to inhibitory levels of LWCA occurred more abruptly in acute assays than in subchronic assays and it was narrower for cress than for ryegrass. In acute assays the interval for cress ranged from 0.5 to 3 mM and for ryegrass from 0.1 to 5 mM. In subchronic assays this interval ranged from 12 to 70 mM kg^{-1} dm for cress and from 16 to almost 200 mM kg^{-1} dm for ryegrass, gradually decreasing from formic to caproic acid. No decreasing trend in the interval magnitude was observed in acute assays.

In subchronic assays cress was more sensitive to LWCA than ryegrass, EC50 values of cress were from 10% to 60% lower than of ryegrass. However, in acute assay the difference between species was not significant for most of the LWCA. As an illustrative example, difference in species sensitivity to caproic acid is compiled in Fig. 3.

Both assays showed general trend of increase in toxicity of LWCA with increase in carbon chain of the molecule, formic or acetic acid being the least toxic and caproic the most. The trend was more obvious in subchronic assays than in acute assays. Order of

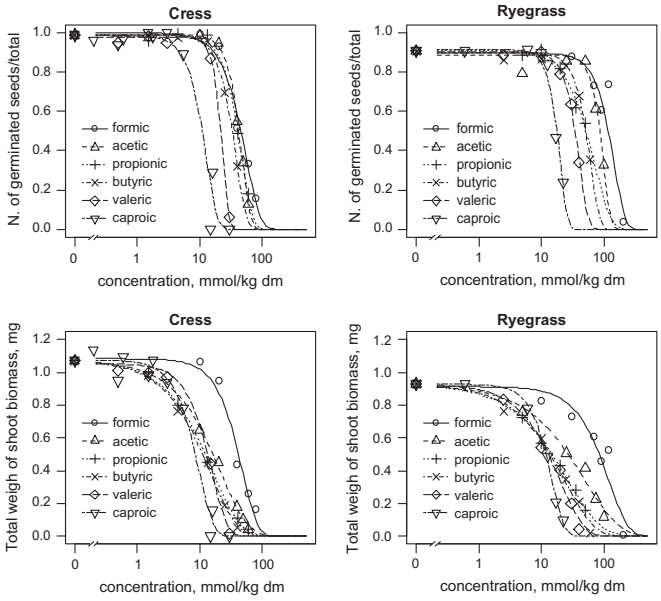

Fig. 2. Dose–response models for LWCA for total germination and 21d growth of shoot mass of *L. sativum* (cress) and *L. multiflorum* (ryegrass) in subchronic assay.

Table 1
Effective concentrations (ECs) of low-weight carboxylic acids (LWCAs) for reduction of seed germination in acute and subchronic assays for cress and ryegrass. EC values were calculated using Weibull 1.3 model and are expressed as averages, with standard errors in parenthesis.

Acid	Acuteassay (mM)		Subchronic assay (mM kg^{-1} dm)	
	Cress EC50 EC10–EC90	Ryegrass EC50 EC10–EC90	Cress EC50 EC10–EC90	Ryegrass EC50 EC10–EC90
Formic	3.6 (0.1) 2.5 (0.1)–4.5 (0.1)	3.7 (0.1) 2.3 (0.2)–5.1 (0.1)	45.9 (2.7) 17.1 (2.8)–86.2 (7.2)	126.5 (6.7) 58.6 (7.5)–206.6 (16.8)
Acetic	4.2 (0.1) 2.5 (0.2)–5.8 (0.1)	6.4 (0.2) 3.8 (0.2)–9.0 (0.5)	42.8 (1.7)[c] 23.5 (3.1)–62.8 (7.2)	90.4 (2.8) 55.1 (5.4)–124.0 (7.9)
Propionic	3.8 (0.1) 2.2 (0.1)–5.2 (0.1)	2.2 (0.1) 1.3 (0.1)–3.0 (0.2)	41.0 (2.3)[c] 19.3 (3.6)–66.3 (5.5)	56.6 (5.1)[d] 18.0 (3.9)–117.4 (28.6)
Butyric	3.1 (0.1) 1.9 (0.1)–4.2 (0.2)	1.8 (0.1)[a] 1.3 (0.1)–2.5 (0.1)	33.3 (1.7) 16.0 (2.5)–53.1 (4.9)	54.4 (2.2)[d] 28.7 (3.8)–81.7 (7.4)
Valeric	2.2 (0.1) 1.6 (0.2)–2.6 (0.1)	1.8 (0.1)[b] 1.1 (0.1)–2.3 (0.9)	22.1 (1.5) 14.6 (1.8)–28.8 (1.9)	36.4 (1.3) 19.7 (3.8)–54.0 (4.6)
Caproic	1.9 (0.0) 1.2 (0.1)–2.5 (0.4)	1.8 (0.1)[a,b] 1.3 (0.1)–2.2 (0.1)	11.1 (1.0) 5.1 (1.2)–18.1 (1.6)	17.5 (0.5) 11.0 (2.5)–50.2 (1.2)

[a,b,c,d] The same letter indicates no statistically significant difference ($p > 0.05$) between EC50 values of the acids in question.

Table 2
Effective concentrations (ECs) of LWCA obtained in acute and subchronic assays for early seedling growth and shoot biomass of cress and ryegrass. EC values were calculated using Weibull 1.3 model and are expressed as averages, with standard error in parenthesis.

Acid	Acuteassay (mM)		Subchronic assay (mMkg^{-1} dm)	
	Cress EC50 EC10–EC90	Ryegrass EC50 EC10–EC90	Cress EC50 EC10–EC90	Ryegrass EC50 EC10–EC90
Formic	2.3 (0.4)[a] 2.1 (1.8)–2.5 (0.6)	1.8 (0.1) 0.9 (01)–2.7 (0.2)	39.5 (2.1) 13.6 (2.0)–77.7 (6.1)	93.2 (6.5) 24.1 (6.1)–220.5 (29.2)
Acetic	2.0 (0.1)[a] 1.3 (0.2)–2.6 (0.1)	2.7 (0.2) 0.9 (0.2)–5.5 (0.6)	14.9 (1.6) 2.2 (0.7)–50.2 (5.7)	31.2 (3.9) 2.8 (1.2)–145.9 (24.1)
Propionic	1.3 (0.1)[b] 0.5 (0.1)–2.2 (0.6)	0.9 (0.3)[d] 0.4 (0.1)–1.4 (0.1)	11.2 (1.2)[e] 1.6 (0.5)–38.2 (6.2)	16.8 (1.7)[f] 1.6 (0.6)–74.1 (12.4)
Butyric	1.1 (0.1)[b,c] 0.4 (0.1)–1.9 (0.3)	0.9 (0.1)[d] 0.3 (0.1)–1.8 (0.2)	10.1 (1.0)[e] 1.5 (0.4)–33.7 (4.2)	15.8 (1.8)[f,g] 2.0 (0.7)–58.6 (7.5)
Valeric	1.0 (0.1)[c] 0.4 (0.1)–1.8 (0.2)	0.9 (0.1)[d] 0.4 (0.1)–1.5 (0.1)	12.9 (1.6) 4.4 (1.6)–25.6 (3.1)	14.3 (1.3)[g] 2.6 (0.7)–42.1 (4.4)
Caproic	0.7 (0.1) 0.2 (0.0)–1.5 (0.2)	1.2 (0.1)[d] 1.1 (1.2)–1.2 (0.6)	8.1 (0.6) 2.9 (0.6)–15.5 (1.8)	12.4 (0.6) 5.1 (0.9)–21.8 (1.5)

[a,b,c,d,e,f,g] The same letter indicates no statistically significant difference ($p > 0.05$) between EC50 values of the acids in question.

increase in toxicity considering statistically significant difference ($p > 0.05$) can be summarized as following:

Seed germination

	Acute	Subchronic
Cress	C2<C3<C1<C4<C5<C6	C1<C2=C3<C4<C5<C6
Ryegrass	C2<C1<C3<C4=C5=C6	C1<C2<C3=C4<C5<C6

Plant development

	Acute (early seedling growth)	Subchronic (shoot biomass)
Cress	C1=C2<C3=C4=C5<C6	C1<C2<C5<C3=C4<C6
Ryegrass	C2<C1<C3=C4=C5=C6	C1<C2<C3=C4=C5<C6

For parameters measured in acute assays EC50 values of the caproic acid were about 30–70% lower than of formic acids, in subchronic assays the difference was about 75–85%. However, for some parameters (germination, early seedling growth and shoot biomass of ryegrass) there was no statistically significant difference in toxicity for C3—C5 acids.

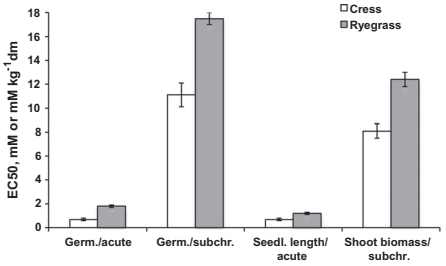

Fig. 3. EC50 values for cress and ryegrass of caproic acid obtained in acute and subchronic assays. Concentrations from acute tests are expressed as mM, from subchronic as mmol kg^{-1} dm. Bars indicate standard errors of the calculated EC50 values.

3.2. pH values and fraction of undissociated LWCA in substrates

In the beginning of the acute assays, pH-values of the solutions varied from 2.9 to 4.6 depending on pK_a and concentration of the LWCA in the solution (supplementary data, Table S2). For comparison, pH of the control was about 5.5. Proportion of undissociated fraction of LWCA in the solutions varied between 0.24 and 0.96.

Table 3
EC50 values for LWCA calculated from dose–response data obtained in different studies. The values were calculated by extrapolation of the original data of dose–response relationships presented by the authors using model Weibull 1.3. No adjustments of pH were made in any of the experiments.

Acid	EC50 values (mM)	Test plant	Reference
Formic			
Germination	4.4	Lettuce (*Lactuca sativa*)	Reynolds (1975)
Seedling growth	0.24	Wheat (*Triticum sp.*)	Prill et al. (1949)
Acetic			
Germination	0.1	Cucumber (*Cucumis sativus*)	Shiralipour and McConnel (1997)
	3.5	Lettuce (*L. sativa*)	Reynolds (1975)
	7.8[a]	Cress (*L. sativum*)	DeVleeschauwer et al. (1982)
	15	Barley *(Hordeum vulgare)*	Lynch (1977)
Seedling growth	0.018	Cucumber (*Cucumis sativus*)	Shiralipour and McConnel (1997)
	1.03	Wheat (*Triticum sp.*)	Prill et al. (1949)
	8.2	Lettuce (*L. sativa*)	Manois et al. (1987)
	10	Barley *(Hordeum vulgare)*	Lynch (1977)
Propionic			
Germination	1.7	Lettuce (*L. sativa*)	Reynolds (1975)
	5	Barley *(Hordeum vulgare)*	Lynch (1980)
Root elongation	0.05	Wheat (*Triticum sp.*)	Prill et al. (1949)
Butyric			
Germination	2.1	Lettuce (*L. sativa*)	Reynolds (1975)
Root elongation	0.08	Wheat (*Triticum sp.*)	Prill et al. (1949)
Valeric			
Germination	1.3	Lettuce (*L. sativa*)	Reynolds (1975)
	4.6	Lettuce (*L. sativa*)	Berrie et al. (1975)
Caproic			
Germination	1	Lettuce (*L. sativa*)	Reynolds (1975)
	2.7	Lettuce (*L. sativa*)	Berrie et al. (1975)
Root elongation	0.06	Wheat (*Triticum sp.*)	Prill et al. (1949)

[a] Concentraion is expressed as mM kg^{-1} dm.

In the beginning of the subchronic assays, pH-values in growth substrates were between 3.0 and 6.7 depending on the concentrations and pK_a of the LWCA (supplementary data, Table S3). However, after the 21 d subchronic exposure, pH-values of the substrates were neutral or slightly basic (pH range from 7.2 to 8.3) in all LWCA, independently of the initial concentrations of the acid. Therefore, at the end of the experiments LWCA were nearly completely dissociated in contrast to the fraction of LWCA in the beginning of the experiments that increased with increase of the acid concentration.

4. Discussion and conclusions

4.1. How strong phytotoxicity of LWCA evoke?

LWCA are important intermediate compounds of aerobic and anaerobic degradation of organic matter, particularly composts (Schlegel, 1986). Despite they are readily biodegradable under aerobic conditions, it may occur that their presence in plant growth substrates may vary when biologically unstable material is applied or due to waterlogging of soils, which may create hypoxic or anaerobic areas between the soil particles. Depending on the microbiological activity LWCA concentrations may rise quickly then and cause damage to plants.

EC50 values obtained in this research in acute assays for seed germination and seedling growth were in a range of concentrations found in the earlier studies. Data from the previous studies with the references are summarized in Table 3. For easier comparison, data were transformed and EC50 values calculated in a similar way as in this research. Depending on the species and endpoint, EC50 values of LWCA range from 0.02 to several mM. EC50 values obtained in this research were on similar level as the values obtained by Reynolds (1975) or Berrie et al. (1975) on lettuce. However, they were about ten times higher than the values reported by Prill et al. (1949) on wheat or hundred times higher reported by Shiralipour and McConnel (1997) on cucumber, though ten times lower than values presented by Lynch (1980) on barley. EC50 values of the subchronic assays were about ten times higher than the values obtained by DeVleeschauwer et al. (1982) on cress or by Chandrasekaran and Yoshida, (1973), who reported a 30–80% decrease in rice seedlings growth caused by 10 mM kg^{-1} of LWCA depending on the acid and soil type. A wide range in EC50 values can be explained to some extent by different sensitivity of test plants to LWCA, physiological differences in seedlings development, the size of endosperm, etc. However, lacking of standardized procedure for toxicological assays can be the main reason for high variability in EC values. Therefore, results of numerical studies conducted so far are difficult to be applied for risk assessment of the treated biogenic materials or in evaluation of LWCA role in substrate phytotoxicity.

In spite of high variability in EC values it is important to mention that concentrations of LWCA, especially acetic acid, equal to EC50 or EC10 can be measured in treated biogenic materials. Concentrations of LWCA can vary from several to tens of mM in bio-waste compost (DeVleeschauwer et al., 1982; Himanen and Hänninen, 2011), anaerobic digestate from manure (Paavola and Rintala, 2008), digestate of organic waste (Tambone et al., 2009). Therefore, these materials might be potentially phytotoxic due to LWCA.

When comparing corresponding EC50 values obtained from acute and subchronic assays, about 10-fold difference between the values can be noticed. Explanation for the difference might lie in degree of dissociation of the LWCA in solution and growth substrate, duration of the assays, partial removal of LWCA due to volatilization or biodegradation or, most probably, a combination of all these factors. Although LWCA concentrations after the experiments were not measured, slightly basic pH of the substrate might indicate decrease in protonic load. Expectedly, LWCA also serve as a carbon and energy sources for micro-organisms (Schlegel, 1986). So, a 21 d assay might have been enough for establishment of the

microbial community and partly degradation of LWCA as the experiments were conducted in clean but not sterile conditions. Volatilization of the LWCA from the substrate could be also possible in spite of the attempts to minimize it. Strong interaction of the LWCA with the substrate particles by sorption due to negatively charged carboxylic groups is strongly possible providing there are positively charged sites (Jones, 1998).

A trend of higher phytotoxicity with increase in molecule chain length was clear, especially with increased duration of the assay. Similar association was suggested in earlier studies for LWCA (Prill et al., 1949; Takijima, 1964; Berrie et al., 1975; Rao and Mikkelsen, 1977; Ulbright et al., 1982), and eventually Marambe et al. (1993) confirmed it by comparing toxicities of LWCA with carbon chain of C2—C10 and fatty acids with carbon chain of C12—C18. The reason for higher toxicity could be increase in lipophilicity at a given pH, thus increasing the bioavailability in the root tissue. According to Lee (1977) toxicity of LWCA, like organic acids in general, are primarily defined by undissociated form of the acids. However, the possible role of anionic species of LWCA in phytotoxicity should be studied more precisely. Another issue that was not addressed in this research, but is important in risk assessment of the treated organic material is the presence of the LWCA as mixtures. Nature of phytotoxicity by LWCA mixtures, whether it is additive, antagonistic or synergistic, is not known.

EC values for six LWCA presented in this paper is a valuable information for evaluation of phytotoxicity potential of the soil substrates or treated biomaterials when they are applied for plant growth. Confirmation of the trend of increased phytotoxicity with molecular size may have more importance for acids with longer carbon skeleton in comparison with shorter ones.

Acknowledgement

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2012.02.058.

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V

LOW-WEIGHT CARBOXYLIC ACIDS AS POTENTIAL RISK IN PHYTOTOXICITY OF PROCESSED BIOMASSES

by

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Low-weight carboxylic acids as potential risk in phytotoxicity of processed biomasses

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ABSTRACT
Being intermediates of organic matter degradation, low-weight carboxylic acids (LWCA) can occur at phytotoxic concentrations in bioactive soils or in the processed organic material applied to soil. While toxicity potentials and mixture effects of LWCA are still poorly known, the potential phytotoxicity of the biomaterials is difficult to assess. In the study effective concentrations (EC) of formic, acetic and propionic acids at non-adjusted pH were obtained in short-term germination assays (48 h for cress, *Lepidium sativum*, and 120h for ryegrass, *Lolium multiflorum*) and subchronic growth assays (21d). Based on the EC values, LWCA inhibited more strongly plant growth than seed germination. In mixtures each acid acted separately without interaction other than additivity. Toxicity of LWCA increased with increase in the carbon chain length, being true also in mixtures. Nontoxic concentration (EC10) for equimolar mixture of formic, acetic and propionic acid was calculated to be below 1000 mg/kg dm.

Key words: mixture phytotoxicity, low-weight carboxylic acids, cress, ryegrass, digestate, compost

1 INTRODUCTION

Low-weight carboxylic acids (LWCA) are intermediates of the natural degradation of organic matter. Although being a pool of transition products, they may accumulate in high concentrations in bioactive natural soils (Stevenson, 1967), the green manures applied to soil due to water logging (Chandrasekaran and Yoshida, 1973) or during processing of biowaste. Analysis of 626 compost samples of different ages showed concentrations of LWCA ranging from 75 to 51474 mg kg^{-1} (Brinton, 1998). In another study concentration range of LWCA in 899 composts was from 72 to 88737 mg kg^{-1} (Brinton and Tränker, 1999). In digestate from organic fraction of municipal solid waste concentration was 18.9 ± 0.5 g acetic acid kg TS^{-1} (Tambone et al., 2009) Concentrations of LWCA in anaerobic effluent from dairy manure ranged from 200 to 800 mg/L depending on the duration of biogas production phase (Rico et al., 2011). Amounts of LWCA in stillage from bioethanol production ranged from 180 to 370 mg/L (Kaparaju et al., 2009). If the processed biomaterial with high concentrations of LWCA is applied for plant production it may exert phytotoxicity, impairing growth of crops (Schuman and McCalla, 1976; Lynch, 1977; Rao and Mikkelsen, 1977; Manois et al., 1987). Thus, although phytotoxicity of LWCA is a known phenomenon, individual effective concentrations (EC) of LWCA are known only to some extent (Himanen et al., 2012). EC values of pure LWCA can be useful in assessment of the phytotoxic potential of treated biowaste, however in the plant growth substrates the acids usually exhibit as a mixture. In a mixture, toxic effect may be predicted by an additive model, where each compound contributes to the toxicity in proportion to its dose. Deviation from additivity may reveal statistically stronger (synergistic) or weaker (antagonistic) outcome (Nielsen et al., 2008). Studies on the mixture of LWCA conducted so far have not been fully systematic. For example, Armstrong and Armstrong (2001) studied effect of two cocktails of LWCA on young common reed *Phragmites*: cocktail 1 (acetic, propionic, n-butyric, iso-butyric, and caproic) and cocktail 2 (formic, acetic, propionic, n-butyric, iso-butyric, valeric and caproic) with concentration of each acid being one mmol/L and pH adjusted to 6. Each cocktail decreased root growth and induced premature shoot senescence. Schuman and McCalla (1976) reported a 70% decrease in root length of wheat and sorghum in a mixture of acetic, propionic and butyric acids over a 40% decrease of individual acids. Although these data are useful in understanding mechanism of LWCA phytotoxicity, however they are not enough to conclude on the type of mixture effect of the acids.

The aim of the present study was to obtain EC values for short-term and subchronic phytotoxicity of formic, acetic and propionic acids and evaluate type of their mixture toxicity using the principle of concentration addition.

2 MATERIALS AND METHODS

Individual and mixture phytotoxicity of formic (F), acetic (A) and propionic (P) acids were studied using short-term (2.2) and subchronic (2.3) assays. Monocotyledonous ryegrass *Lolium multiflorum* and dicotyledonous cress *Lepidium stivum* were used as test plants.

2.1 Concentrations of acids in assays

For individual toxicity a series of pure acids with five concentrations and control were used (Table 1). Choice of the concentrations was based on the preliminary range-finding experiments. Phytotoxicity of binary (F+A, F+P, A+P) and ternary (F+A+P) mixtures of LWCA was studied using the principle of concentration addition. Binary and ternary mixtures were prepared using proportions of the respective EC50-values (=1 toxic unit (TU)) obtained in the preliminary individual toxicity assays. Due to high variability in seed germination, EC50 values for seedling or biomass growth were chosen as basis for the TU concentrations. Summed concentrations of the binary mixtures in short-term and subchronic assays were: ∑ 0.1 TU, ∑ 0.25 TU, ∑ 0.5 TU, ∑ 1 TU, ∑ 2 TU, ∑ 4 TU and in the ternary mixtures: ∑ 1/12 TU, ∑ 1/6 TU, ∑ 1/3 TU, ∑ 1½ TU, ∑ 3 TU. Procedures for conducting the short-term and subchronic assays are described in ch. 2.2 and 2.3.

Table 1: Concentrations of formic, acetic and propionic acids applied in short-term and subchronic assays for individual phytotoxicity studies.

		Cress					Ryegrass				
Short-term assay (mmol/L)											
Formic	Control[a]	0.5	1	2	4	12	0.4	0.8	1.6	3.2	9.6
Acetic	Control	0.5	1	1.5	3	12	0.4	0.8	1.2	2.4	9.6
Propionic	Control	0.3	0.6	1.2	2.4	9.6	0.3	0.6	1.2	2.4	9.6
Subchronic assay (mmol/kg dm)											
Formic	Control[b]	5	10	20	40	80	10	30	60	120	150
Acetic	Control	5	10	20	40	80	5	25	50	75	100
Propionic	Control	2	5	10	30	60	5	10	15	30	60

[a] de-ionized water

[b] de-ionized water added to inorganic growth substrate

2.2 Short-term phytotoxicity assays

For short-term assays Petri dishes (∅ 90 mm) were lined with the filter paper (Whatman no.1, ∅ 70 mm) and moisturized with 10 mL of pure acid solution, mixture of LWCA or control (de - ionized water) (see ch. 2.1). Each plate was seeded with 20 seeds of garden cress *L. sativum* (seed supplier Habitec Inc., Finland) or ryegrass *L. multiflorum v. fabio* (seed supplier Tilasiemen OY, Finland) and covered with the lid. Petri dishes were incubated in the darkness at 24–26 °C for 48 (garden cress) or 120 hours (ryegrass). After the incubation, number of the germinated seeds was counted and total length of the seedlings (root + shoot) for garden cress or the longest root for ryegrass was measured with the accuracy of 1 mm. The seed was considered to be germinated when radicle was over 1 mm long. One set of the experiment consisted of three Petri dishes for each concentration and six Petri dishes for control repeated three times. An experiment was considered successful when germination of controls of cress was over 95% and that of ryegrass over 85%.

2.3 Subchronic phytotoxicity assay

The subchronic assays were conducted according to the modified standard method ISO 11269-2 (ISO, 2008) with garden cress *L. sativum* and ryegrass *L. multiflorum v. fabio.* Five dilutions were prepared for each pure acid or LWCA mixture (see ch. 2.1). The inorganic growth substrate was made of the coarse-grained sand (particle size 0.5–1.2 mm; Maxit Inc., Finland) and the quartz sand (particle size 0.2–0.005 mm; NFQ Nilsiän kvartsi, SP Minerals Inc., Finland) in proportion 6+1 (v/v). The contamination of the growth substrate was made by adding 100 ml of pure acid, LWCA mixture solution, or control (de-ionized water) to 1.6 L of the growth substrate. The plastic pot (∅ = 10 cm, height = 8 cm) was filled as following: approx. one cm height of peat-based growing media (Kekkilä Kasvuturve B2, Kekkilä Inc., Finland) on the bottom, 300 g of the inorganic substrate, 25 seeds, and approx. one cm of the peat-based growing media on the top. Peat-based layers were used to prevent leaching of the substrate from the pot during watering and to decrease stripping of the acids from the substrate. The pots were incubated in a climate room at 23–27 °C with light/dark regime 16/8 hours, the light being 13 000 lx, 185 µmol m^{-2} s^{-1}, with the color temperature of 6500 K (Biolux, Osram, Germany). To avoid drying of the substrate, the plants were watered with general fertilizing solution (NPK 12–6–9, Kekkilä Kukkaravinne, Kekkilä Inc., Finland) every or every second day. Incubation time was 21 days, after which the number of germinated plants was counted, the plants were cut next to the substrate surface, dried at 70 °C over night and weighted. Each experiment consisted of six control replicates and three replicates for each acid or their mixture concentration, repeated three times.

2.4 Modeling dose–response relationships

Modeling of dose–response relationships for each endpoint (germination, early seedling growth and shoot biomass) was done using added concentrations of LWCA as actual concentrations in the substrates were not analyzed. Modeling and calculations of the EC-values and statistical parameters were done for each acid and LWCA mixture using R program (version 2.10.1) drc-package. Different models were tested and the three-parameter log-logistic model was used for the final data extrapolation. The model is applicable when the response pattern follows a non–linear curve, it is symmetric around EC50 point, and the lower limit is equal to zero. The model is described by equation 1.

$$f(x, (b, d, e)) = d / (1 + \exp\{b (\log (x) - \log (e))\}) \quad (1)$$

The parameter d is the lower limit, b is the relative slope around e, and e is the logarithm of the inflection point (Ritz and Streibig, 2005).

2.5 Toxicity analysis of LWCA mixtures

Toxicity analysis of the mixtures was made using the interaction index according to Marking (1985). For that, the sum of toxic action (S) and the additive index (AI) were calculated using equations 2–4.

$$S = (A_m/A_i) + (B_m/B_i) + (C_m/C_i) \quad (2)$$

S = sum of toxic action; A_m, B_m, C_m = EC50 for compounds A, B and C in mixtures; A_i, B_i, C_i = EC50 for compounds A, B and C individually. S values were used for calculation of the AI.

$$If\ S \leq 1.0,\ AI = (1/S) - 1.0 \quad (3)$$
$$If\ S \geq 1.0,\ AI = S\ (-1) + 1 \quad (4)$$

The AI significantly less than zero indicates antagonistic toxicity and greater than zero synergistic toxicity. The significance of deviation from zero was evaluated by generating confidence interval (CI) values for AI by substituting the EC50 values in equation 1 with the corresponding CI limit values of EC50 according to equations 5 and 6.

$$AI_{m(low)} = (CI_{A\ m(up)}/CI_{A\ i\ (low)}) + (CI_{B\ m(up)}/CI_{B\ i\ (low)}) + CI_{C\ m(up)}/CI_{C\ m(low)}) \quad (5)$$
$$AI_{m(up)} = (CI_{A\ m(low)}/CI_{A\ i(up)}) + (CI_{B\ m(low)}/CI_{B\ i(up)}) + CI_{C\ m(low)}/CI_{C\ m(up)}) \quad (6)$$

where $AI_{m(low)}$ *and* $AI_{m(up)}$ = lower and upper limits of the AI interval, $CI_{A/B/C\ m(up)}$ *and* $CI_{A/B/C\ m(low)}$ = upper and lower limits of EC50 confidential intervals obtained for the

compounds A, B, and C in mixture assays; $CI_{A/B/C\ i(up)}$ *and* $CI_{A/B/C\ i(low)}$ = upper and lower limits of EC50 confidential intervals obtained for the compounds A, B, and C in individual assays. If AI confidential interval overlaps zero, mixture can be judged to have additive toxicity (Marking, 1985).

3 RESULTS AND DISCUSSION

3.1 Toxicity of individual acids

Dose–response relationships of formic, acetic and propionic acids for seed germination, early seedling growth, and plant growth were modeled (Figs. 1 and 2) and the models were used to calculate effective concentration values such as EC10, EC50, and EC90 for each LWCA (Table 2).

Results showed ryegrass being more sensitive to LWCA than cress. For seed germination obtained in short-term assays, EC50 values for formic, acetic and propionic acids were between 2.9 and 4.4 mmol/L for cress and between 1.8 and 3.6 mmol/L for ryegrass. In subchronic assays EC50 values for delayed germination of cress were between 25 and 37 mmol/kg dm, and of ryegrass 35 and 78 mmol/kg dm. Regarding to the early seedling growth EC50 values for cress were between 1.1 and 2.0 mmol/L and for ryegrass between 1.8 and 2.4 mmol/L. EC50 values for plant biomass of cress were between 12 and 36 mmol/kg dm, and of ryegrass between 16 and 50 mmol/kg dm. Variability of data was similar in both tests, relative standard error (RSE) was between 3 and 13%.

Considering EC10–EC90 intervals, the transition from non-toxic to inhibitory levels of LWCA occurred more abruptly in short-term than in subchronic assays being narrower for cress than for ryegrass. In short-term assays the interval for all endpoints for cress ranged from 2 to 4 mmol/L and for ryegrass from 3 to 7 mmol/L. In subchronic assays this interval ranged from 23 to 33 mmol/kg dm for cress and from 22 to almost 80 mmol/kg dm for ryegrass. Thus, in subchronic assays, cress was somewhat more sensitive to LWCA than ryegrass, EC50 values of cress were by 30 to 75 % lower than of ryegrass. However, in short-term assays the difference between species was not significant.

EC values obtained in the short-term assays of this study were on the same level as found by Reynolds (1975) on lettuce and Himanen et al. (2012) on cress and ryegrass. However, current values were about ten times higher than the values reported by Prill et al. (1949) on wheat or hundred times higher reported by Shiralipour and McConnel (1997) on cucumber. Though, the EC values on barley presented by Lynch (1977 and 1980) were three to five times lower than the values in this research. In subchronic assays, the EC values in this research were by 15–40% lower than obtained by Himanen et al. (2012) on ryegrass and cress.

Phytotoxicity of LWCA increased with the length of the carbon chain that was more clearly observed in subchronic assays than in short-term assays, which is compatible with the earlier observations (Prill et al., 1949; Ulbright et al., 1982; Himanen et al., 2012).

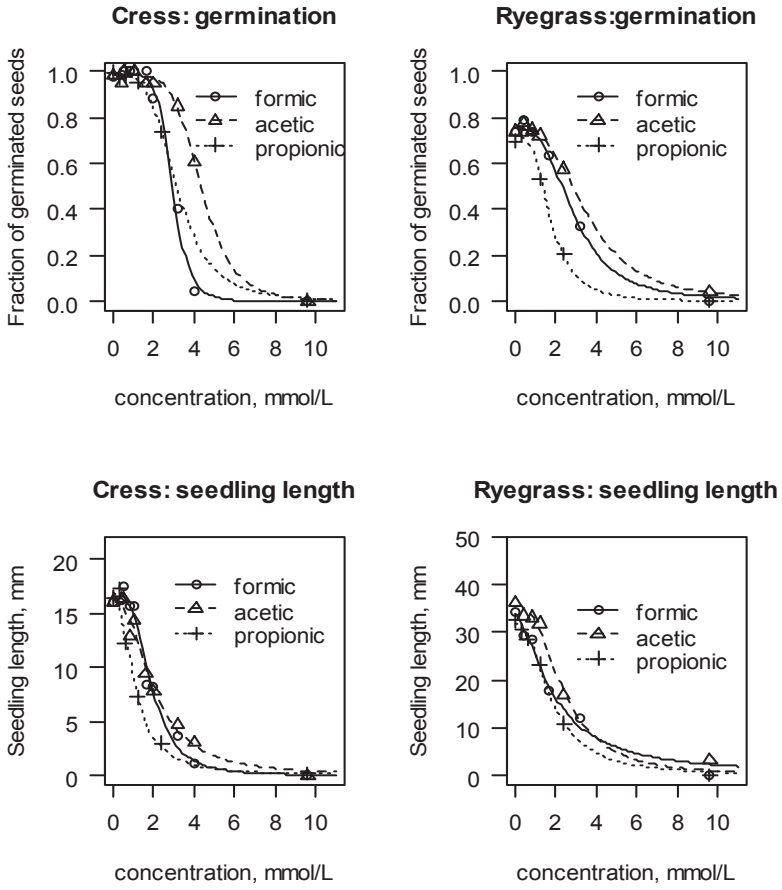

Figure 1: Dose-response curves of individual formic, acetic and propionic acids for germination and seedling growth of cress and ryegrass obtained in short-term assays.

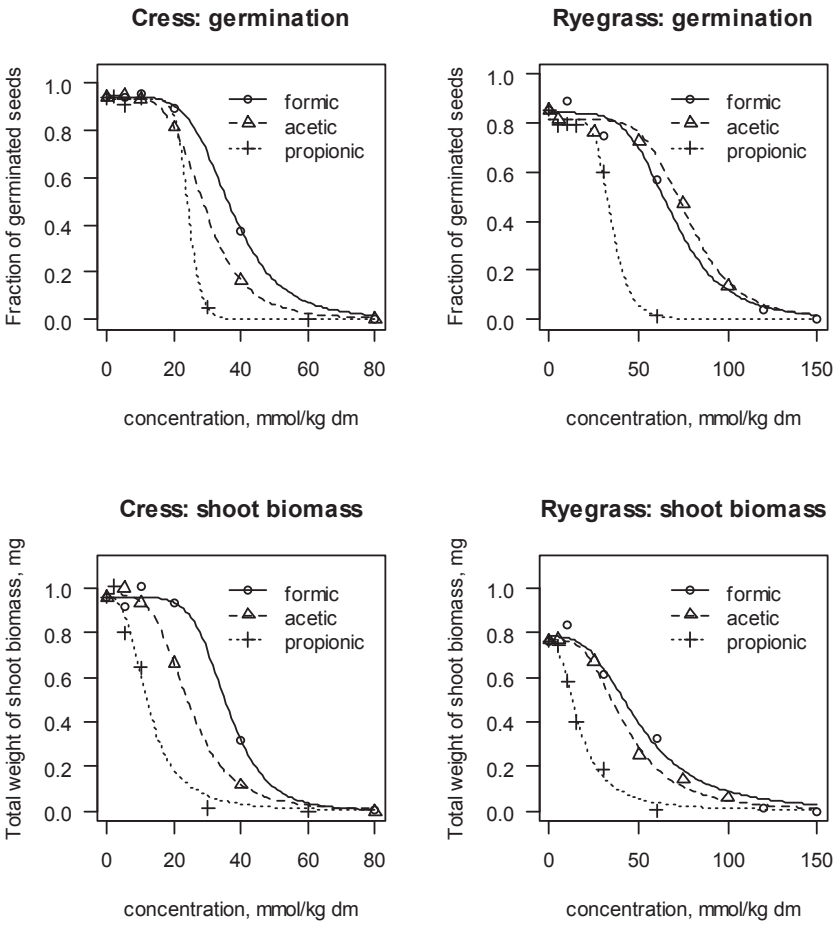

Figure 2: Dose-response curves of individual formic, acetic and propionic acids for germination and seedling growth of cress and ryegrass obtained in subchronic assays.

Table 2: Effective concentrations (EC) of formic, acetic and propionic acids, assayed individually, for reduction of seed germination, seedling growth, and shoot biomass obtained in short-term and subchronic assays. Test species were cress *Lepidium sativum* and ryegrass *Lolium multoflorum*. EC values are expressed as means with standard errors in parenthesis.

	Short-term assay (mmol/L)		Subchronic assay (mmol/kg dm)	
	Cress	Ryegrass	Cress	Ryegrass
	EC50	EC50	EC50	EC50
	EC10 - EC90	EC10 - EC90	EC10 - EC90	EC10 - EC90
	Germination			
Formic	2.9 (0.1)	2.9 (0.2)	37 (1.2)	69 (2.3)
	2.2 (0.1) - 4.0 (0.1)	1.4 (0.2) - 6.0 (0.1)	24 (2.8) - 57 (4.5)	44 (4.0) - 108 (11.4)
Acetic	4.4 (0.1)	3.6 (0.5)	29 (1.1)	78 (2.1)
	3.0 (0.2) - 6.4 (0.7)	1.7 (0.3) - 7.5 (2.1)	19 (1.2) - 46 (2.4)	53 (4.3) - 114 (7.1)
Propionic	3.2 (0.2)	1.8 (0.1)	25 (NA*)	35 (2.3)
	1.8 (0.1) - 5.6 (1.0)	0.9 (0.1) - 3.5 (0.5)	21 (NA) - 30 (NA)	25 2.2) - 47 (9.3)
	Seedling growth		Shoot biomass	
Formic	1.9 (0.1)	1.9 (0.2)	36 (2.9)	50 (4.6)
	1.0 (0.1) - 3.7 (0.3)	0.5 (0.1) - 7.5 (1.9)	25 (7.0) - 51 (7.4)	24 (4.7) - 104 (14.5)
Acetic	2.0 (0.1)	2.4 (0.2)	24 (1.7)	42 (3.3)
	0.8 (0.1) - 5.2 (0.5)	1.0 (0.3) - 5.8 (2.1)	14 (1.9) - 42 (6.3)	21 (3.8) - 87 (11.6)
Propionic	1.1 (0.1)	1.8 (0.2)	12 (1.3)	16 (1.6)
	0.4 (0.0) - 3.0 (0.3)	0.7 (0.2) - 4.9 (1.3)	5 (1.2) - 28 (6.0)	6 (1.3) - 43 (8.4)

*NA - Data could not be generated due to lack of data between 100% germination (10 mmol/kg dm) and no germination (30 mmol/kg dm), that defined almost vertical slope around EC50 value.

3.2 Mixture toxicity of LWCA

In binary mixture assays for all endpoints (germination, early seedling growth and biomass production) EC50 values were around 1 TU, the means ranging from 0.8 to 1.4

TU for F+A mixture and from 0.6 to 1.2 TU for F+P and A+P mixtures (Table 3). In ternary mixture EC50 in short term assays was slightly lower (range between 0.4 and 1.0 TU) than in subchronic assays (range between 0.9 and 1.3 TU).

Table 3: EC50 values and 95% confidential intervals (in parenthesis) generated from the data obtained in short-term and subchronic assays for binary and ternary mixtures of formic (F), acetic (A) and propionic (P) acids. The values are expressed as toxic units and were extrapolated from three-parametric log-logistic model.

	Short-term assay		Subchronic assay	
	Cress	Ryegrass	Cress	Ryegrass
	Germination		Germination	
F + A	1.2	1.4	0.9	1.1
	(1.2–1.3)	(1.2–1.5)	(0.8–0.9)	(1.0–1.2)
F + P	1.2	1.0	1.3	1.1
	(1.2–1.2)	(1.0–1.2)	(1.2–1.4)	(1.0–1.3)
A + P	1.2	1.0	0.9	0.7
	(1.1–1.3)	(1.0–1.1)	(0.8–1.0)	(0.6–0.8)
F+A+P	1.0	0.7	1.1	1.3
	(1.0–1.0)	(0.6–0.9)	(1.1–1.2)	(1.2–1.4)
	Seedling growth		Shoot biomass	
F + A	0.8	1.5	0.9	0.8
	(0.7–0.9)	(1.2–1.7)	(NA*–NA)	(0.6–1.0)
F + P	0.6	0.8	1.0	0.9
	(0.5–0.6)	(0.7–1.0)	(0.8–1.1)	(0.6–1.2)
A + P	0.6	0.1	0.6	0.6
	(0.5–0.6)	(0.8–1.2)	(0.5–0.7)	(0.5–0.8)
F+A+P	0.4	0.7	0.9	1.0
	(0.4–0.5)	(0.5–0.9)	(0.7–1.1)	(0.8–1.2)

*NA – the values of the confidential interval could not be generated.

For most endpoints interaction index of binary mixtures analysis showed additive value around one (Tables 4 and 5). The range of the index limit values was slightly negative or overlapping zero, meaning no other type of interactions like synergistic or antagonistic effects. So, toxic unit and interaction index analysis suggested simple dose addition mechanism of the LWCA in mixtures. This means that in a mixture each acid acts separately and no interactions occur between the acids. Therefore, phytotoxicity of the substrate depends on what LWCA are present in the substrate and is proportional to concentration of each acid. As LWCA belong to the same group of carboxylic acids they possibly act similarly on plants. Mechanisms of LWCA phytotoxicity is not clearly understood, yet. There is no common agreement whether dissociated or undissociated form of LWCA is phytotoxic. The toxicity of LWCA can be regarded as partly due to

H^+-ion and partly due to undissociated acid or dissociated anion (Stiles and Rees, 1935). One possible way of LWCA toxic action is disturbance of osmotic regulations of root cells. Once the molecules have entered the root cells, they change permeability of the membrane and cause hyperpolarization of transmembrane electrical potential. This leads to rapid loss of K^+, Ca^{2+}, and Cl^- ions into the external substrate and flow of Na^+ ions into the cell that was demonstrated in many experimental studies (Jackson and Taylor, 1970; Lee, 1977; Marrè et al., 1983).

Role of the carbon chain length becomes more important in mixture toxicity, i.e. mixture of acids with longer chain is more phytotoxic than mixture of acids with shorter chain. For example, in short-term responses for cress, EC50 value of acetic acid alone was 2.0 mmol/L (95% conf.int 1.8–2.2), which is higher than 1.0 mmol/L (1.0–1.1) in A+F, 0.8 mmol/L (0.7–0.8) in A+P and 0.6 mmol/L (0.5–0.6) in F+A+P. However, the trend was not so obvious in subchronic endpoints. As an example, graphical comparison of EC50 values from individual and mixture assays for cress from short-term and subchronic assays are presented in Figure 3. Increase of toxicity in mixture compared to the pure acids was observed for acetic, propionic and butryric acids by Shuman and McCalla (1976). In the study germination of wheat in the equimolar mixture was 51% compared to toxicity of pure acids that was 73-89%, for sorghum respective values were 49% and 53–67%. The trend may suggest that in a mixture each LWCA has its own relative potency (REP) value that increases with the increase of the carbon chain. Similar suggestion was made by Lynch (1977) stating that at equivalent concentrations, the phytotoxicity of propionic and butyric acids was greater than that of acetic acid by factors of about 2 and 3, respectively. If this hypothesis is true propionic and butyric acids still make a contribution to the phytotoxicity of compost, although they present in small concentrations. However, more research is needed following also phytotoxic effect of C4–C6 LWCA.

Table 4: EC50 values, confidential intervals (95% CI), the sums of toxic action (S) and the additive indices (AI) for individual and mixture toxicity of formic (F), acetic (A) and propionic (P) acids obtained in short-term germination assays for cress and ryegrass. Concentrations are expressed in mmol/L.

	Cress		Ryegrass	
	EC50	95% CI	EC50	95% CI
GERMINATION				
		Formic + Acetic		
Formic individual	2.9[a]	2.8 – 3.1	2.9	2.5 – 3.3
Formic in F + A	2.0[b]	1.9 – 2.1	2.0	1.8 – 2.2
Acetic individual	4.4	4.1 – 4.6	3.6	2.7 – 4.5
Acetic in F + A	1.6	1.5 – 1.7	3.0	2.7 – 3.3
S		1.1 (1.2; 1.0)[c]		1.5 (2.1; 1.2)
AI		-0.1 (-0.2; 0.1)[d]		-0.5 (-1.1; -0.2)
		Formic + Propionic		
Formic individual	2.9	2.8 – 3.1	2.9	2.5 – 3.3
Formic in F + P	1.9	1.8 – 2.0	1.5	1.3 – 1.7
Propionic individual	1.8	1.5 – 2.0	1.8	1.5 – 2.0
Propionic in F + P	1.2	1.2 – 1.2	1.3	1.1 – 1.5
S		1.0 (1.2; 0.9)		1.3 (1.7; 1.0)
AI		0.0 (-0.2; 0.1)		-0.3 (-0.7; 0.0)
		Acetic + Propionic		
Acetic individual	4.4	4.1 – 4.6	3.6	2.7 – 4.5
Acetic in A + P	1.6	1.5 – 1.6	2.2	2.1 – 2.4
Propionic individual	1.8	1.5 – 2.0	1.8	1.5 – 2.0
Propionic in A + P	1.2	1.1 – 1.3	1.3	1.2 – 1.4
S		0.7 (0.9; 0.6)		1.4 (1.8; 1.1)
AI		0.4 (0.2; 0.6)		-0.4 (-0.8; -0.1)
		Formic + Acetic + Propionic		
Formic in F+A+P	1.6	1.5 – 1.6	1.1	0.9 – 1.3
Acetic in F+A+P	1.3	1.2 – 1.3	1.6	1.3 – 1.9
Propionic in F+A+P	1.0	1.0 – 1.0	0.9	0.8 – 1.1
S		1.39 (1.6; 1.2)		1.4 (2.0; 0.9)
AI		-0.4 (-0.6; -0.2)		-0.4 (-1.0; 0.07)

SEEDLING GROWTH				
	Formic + Acetic			
Formic individual	1.9	1.8 – 2.1	1.9	1.4 – 2.4
Acetic individual	2.0	1.8 – 2.2	2.4	1.9 – 2.9
Formic in F + A	1.3	1.2 – 1.4	2.4	2.0 – 2.8
Acetic in F + A	1.0	0.9 – 1.1	1.9	1.6 – 2.2
S		1.2 (1.4; 1.0)		2.0 (3.1; 1.4)
AI		-0.2 (-0.4; 0.0)		-1.0 (-2.1; -0.4)
	Formic + Propionic			
Formic individual	1.9	1.8 – 2.1	1.9	1.4 – 2.4
Formic in F + P	0.9	0.9 – 1.0	1.3	1.1 – 1.5
Propionic individual	1.1	1.0 – 1.2	1.8	1.4 – 2.2
Propionic in F + P	0.6	0.5 – 0.6	0.8	0.7 – 1.0
S		1.0 (1.2; 0.9)		1.2 (1.8; 0.8)
AI		0.0 (-0.2; 0.1)		-0.2 (-0.8; 0.2)
	Acetic + Propionic			
Acetic individual	2.0	1.8 – 2.2	2.4	1.9 – 2.9
Acetic in A + P	0.8	0.7 – 0.8	1.3	1.1 – 1.5
Propionic individual	1.1	1.0 – 1.2	1.8	1.4 – 2.2
Propionic in A + P	0.6	0.5 – 0.6	1.0	0.8 – 1.2
S		0.9 (1.1; 0.8)		1.1 (1.6; 0.7)
AI		0.1 (-0.1; 0.3)		0.0 (-0.6; 0.3)
	Formic + Acetic + Propionic			
Formic in F+A+P	0.7	0.6 – 0.8	1.0	0.7 – 1.4
Acetic in F+A+P	0.6	0.5 – 0.6	1.5	1.0 – 2.0
Propionic in F+A+P	0.4	0.4 – 0.5	0.9	0.6 – 1.2
S		0.6 (0.7; 0.5)		1.3 (0.7; 2.0)
AI		0.7 (0.4; 1.0)		-0.3 (-1.0; 0.4)

[a] calculated by extrapolation of the data from the assays on pure acid

[b] converted from TU values that were obtained by extrapolation of the data from mixture assays

[c] the sum of toxic action calculated according to eq. 2 with 95% confidential interval in parenthesis

[d] additive index, calculated according to eq. 3 or 4, with 95% confidential interval in parenthesis calculated according to eq. 5 and 6.

Table 5: EC50 values, confidential intervals (95% CI), the sums of toxic action (S) and the additive indices (AI) of individual and mixture toxicity of formic (F), acetic (A) and propionic (P) acids obtained in subchronic plant growth assays for cress and ryegrass. Concentrations are expressed in mmol/kg dm.

	Cress		Ryegrass	
	EC50	95% CI	EC50	95% CI
GERMINATION				
	Formic + Acetic			
Formic individual	37[a]	35 – 39	69	64 –74
Formic in F + A	31[b]	28 – 35	56	51 – 61
Acetic individual	29	35 – 39	78	73 – 82
Acetic in F + A	25	18 – 22	47	43 – 51
S		1.7 (1.8; 1.3)[c]		1.4 (1.7; 1.2)
AI		-0.7 (-0.8; -0.3)[d]		-0.4 (-0.7; -0.2)
	Formic + Propionic			
Formic individual	37	35 – 39	69	34 – 74
Formic in F + P	47	43 – 52	57	49 – 64
Propionic individual	25	27 – 31	35	30 – 39
Propionic in F + P	14	13 – 16	18	16 - 21
S		1.8 (2.1; 1.5)		1.4 (1.7; 1.1)
AI		-0.8 (-1.1; -0.5)		-0.4 (-0.7; -0.1)
	Acetic + Propionic			
Acetic individual	29	35 – 39	78	73 – 82
Acetic in A + P	32	29 –35	31	27 – 34
Propionic individual	25	27 – 31	35	30 – 39
Propionic in A + P	10	9 – 11	12	10 – 13
S		1.5 (1.7; 1.2)		0.7 (0.9; 0.6)
AI		-0.3 (-0.4; -0.2)		0.4 (0.1; 0.7)
	Formic + Acetic + Propionic			
Formic in F+A+P	41	39 – 43	64	59 – 69
Acetic in F+A+P	27	26 – 28	54	50 – 58
Propionic in F+A+P	12	12 – 13	20	19 – 22
S		2.5 (2.8; 2.2)		2.2 (2.6; 1.9)
AI		-1.5 (-1.8; -1.2)		-1.2 (-0.9; -1.6)

SHOOT BIOMASS				
	Formic + Acetic			
Formic individual	36	30 – 41	50	41 – 59
Acetic individual	32	NA – NA	40	31 – 48
Formic in F + A	24	21 – 27	42	36 – 49
Acetic in F + A	22	NA – NA	33	27 – 40
S		1.8 (NA;NA)		1.6 (2.3; 1.1)
AI		0.8 (NA; NA)		-0.6 (-1.3; -0.1)
	Formic + Propionic			
Formic individual	36	30 – 41	50	41 – 59
Formic in F + P	34	28 – 41	45	30 – 59
Propionic individual	12	10 – 15	16	13 – 19
Propionic in F + P	11	9 – 12	14	10 – 19
S		1.8 (2.6; 1.3)		1.8 (2.9; 1.0)
AI		-0.8 (-1.6; -0.3)		-0.8 (-1.9; 0.0)
	Acetic + Propionic			
Acetic individual	24	21 – 27	42	36 – 49
Acetic in A + P	15	12 – 18	27	22 – 32
Propionic individual	12	10 – 15	16	13 – 19
Propionic in A + P	7	6 – 8	10	8 – 12
S		1.2 (1.7; 0.8)		1.3 (1.8; 0.9)
AI		-0.2 (-0.7; 0.2)		-0.9 (-0.8; 0.1)
	Formic + Acetic + Propionic			
Formic in F+A+P	31	24 – 38	50	40 – 60
Acetic in F+A+P	21	16 – 25	42	34 – 51
Propionic in F+A+P	9	7 –12	16	13 – 19
S		2.5 (3.7; 1.6)		3.0 (4.3; 2.0)
AI		-1.5 (-0.6; -2.7)		-2.0 (-1.0; -3.3)

[a] calculated by extrapolation of the data from the assays on pure acid

[b] converted from TU values that were obtained by extrapolation of the data from mixture assays

[c] the sum of toxic action calculated according to eq. 2 with 95% confidential interval in parenthesis

[d] additive index, calculated according to eq. 3 or 4, with 95% confidential interval in parenthesis calculated according to eq. 5 and 6.

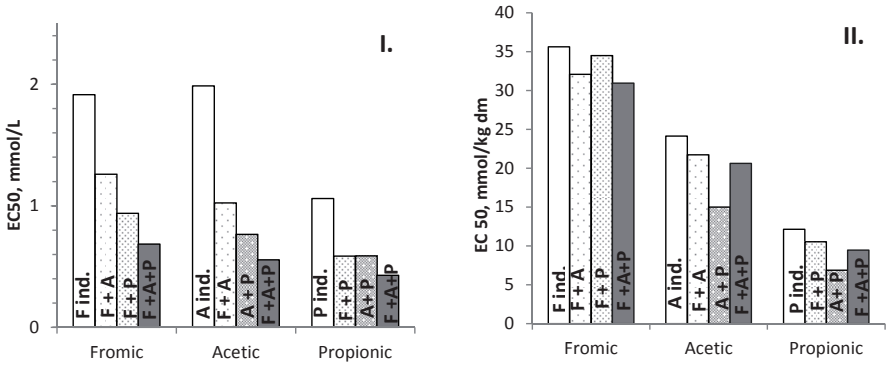

Figure 3: Comparative example of individual and mixture EC50 values of formic (F), acetic (A) and propionic (P) acids obtained in short-term (I) and subchronic (II) assays for growth of cress. EC50 values in I are for seedling length and in II for dry biomass.

3.3 Phytotoxic levels of LWCA in processed biomasses

For utilization of the results in evaluation of the toxicity potential of growth substrates, it might be more practical to use values of EC10 or EC20 instead of EC50. EC10 and EC20 indicate a 10% or 20% decrease in plant production compared to the experimental control and can be considered as near nonphytotoxic. For example, according to a national decree 1784/14/2011 on fertilizers issued by the Finnish Ministry of Forestry and Agriculture, a 20% decrease in germination index of cress is acceptable for soil improvers produced from treated organic material. Based on the present study, for formic, acetic and propionic acids, mixed in equal proportions, EC10 of LWCA mixture would be around 15 mmol/kg dm (900 mg/kg dm) for cress and 17 mmol/kg dm (1000 mg/kg dm) for ryegrass. Generalizing, we may suggest a nontoxic concentration of LWCA to be some less than 1000 mg/kg dm. The value is close to the concentration of 1250 mg/kg of total LWCA in compost-containing growth media suggested by Brinton and Tränkner (1999). Additionally it is important to mention, that in treated organic materials acetic acid is usually dominating over the other LWCA (DeVleeschauwer et al., 1982; Himanen and Hänninen, 2011). In such cases, EC values of the acetic acid would practically define phytotoxic level of the substrate. However, if REP values for LWCA can be estimated, their contribution to the phytotoxicity of substrate should be taken into consideration even if they present in low concentrations.

In order to decrease levels of LWCA in treated biomaterials and, thus, decrease phytotoxicity of the substrate, volatility and biodegradability features of the acids may be utilized. In practice it means that mixing of the mass and improved aeration will increase evaporation of LWCA from the mass and better oxygen supply enhances oxidative microbial degradation. However, stripping of LWCA from the mass will increase offensive odors, which may add to nuisance problems. By increasing duration of the maturation stage, slow degradation of LWCA may be achieved and thus decrease the phytotoxicity as well.

4 CONCLUSIONS

The research aimed to study individual and mixture phytotoxicity of formic, acetic and propionic acids by short-term and subchronic assays. According to the EC values, phytotoxicity of LWCA increases with the length of carbon chain, which is also true in mixtures. In mixtures LWCA act according to the dose addition mechanism, suggesting each acid acts separately and no other type of interaction occurs between the acids. Thus, knowing concentration of each acid in a plant growth substrate, total phytotoxicity can be evaluated, e.g. nontoxic concentration (EC10) for equimolar mixture of formic, acetic and propionic acid is below 1000 mg/kg dm.

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