Eesti Maaülikool
Estonian University of Life Sciences

# BIO-WASTE COMPOSTING IN THE FRAMEWORK OF A CIRCULAR ECONOMY

# BIOJÄÄTMETE KOMPOSTIMINE RINGMAJANDUSE TINGIMUSTES

**MARGE LANNO**

A Thesis
for applying for the degree of Doctor of Philosophy
in Agriculture

Väitekiri
filosoofiadoktori kraadi taotlemiseks
põllumajanduse erialal

Tartu 2023

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# LIST OF ORIGINAL PUBLICATIONS

The present thesis is based on the following research papers, which are referred to by their Roman numerals (**I–III** in the text). Papers are reproduced by kind permission of the journals concerned.

I **Lanno, M.**, Silm, M., Shanskiy, M., Kisand, A., Orupõld, K., & Kriipsalu, M. (2020). Open windrow composting of fish waste in Estonia. Agronomy Research, 18 (4), 2465−2477. doi: 10.15159/AR.20.194.

II **Lanno, M.**, Kriipsalu, M., Shanskiy, M., Silm, M., & Kisand, A. (2021). Distribution of Phosphorus Forms Depends on Compost Source Material. Resources, 10 (10), ARTN 102. doi: 10.3390/resources10100102.

III **Lanno, M.**, Klavins, M., Purmalis, O., Shanskiy, M., Kisand, A., & Kriipsalu, M. (2022). Properties of Humic Substances in Composts Comprised of Different Organic Source Material. Agriculture, 12(11), 1797. https://doi.org/10.3390/agriculture12111797

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| II | **ML** AK $MK^2$ $MS^1$ | **ML** AK | **ML** AK | **ML** $MK^2$ $MS^1$ $MS^2$ AK |
| III | **ML** $MK^1$ OP $MS^1$ AK $MK^2$ | **ML** $MK^1$ $MK^2$ OP | $MK^1$ OP | **ML** $MK^1$ OP $MS^1$ AK $MK^2$ |

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# ABBREVIATIONS

| | |
|---|---|
| SDG | Sustainable Development Goals |
| GHG | Greenhouse gases |
| ABPR | Animal By-Products |
| Labile-P | Dissolved and loosely bound P |
| Fe-P | P bound to reducible metals (Fe, Mn) |
| Al-P | P bound to aluminum (Al) |
| Ca-P | P bound to calcium (Ca) |
| Org-P | P bound to organic material and colloids, determined as difference between soluble reactive P and total P in NaOH extract |
| SumP | Summary of P fractions (Labile-P, Fe-P, Al-P, Ca-P, Org-P) |
| TotP | Total P |
| ResP | Residual P, difference between TotP and SumP |
| DW | Dry weight (g) |
| SOM | Soil organic matter |
| HS | Humic substances |
| HA | Humic acids |
| FA | Fulvic acids |
| TOC | Total organic carbon |
| FTIR | Fourier-transform infrared |
| UV-Vis | Ultraviolet-visible |
| EEM | Excitation Emission Matrix |
| WWTP | Wastewater treatment plant |

# 1. INTRODUCTION

Growing population and changing climate increases the concerns about food security and price stability (Schmidhuber & Tubiello, 2007). It has been estimated that if no action is taken, global temperatures can increase 1.5 °C compared to pre-industrialised levels in the next decade due to climate change. This shift will change environmental conditions and agricultural production. Temperature changes can, in some areas, expand the growing period and increase crop yields. However, more frequent storms, droughts, and other extreme weather conditions are also projected to occur (European Commission, 2019; IPCC, 2023).

Another threat to global food security is widespread usage of mineral fertilizers. Overuse of mineral fertilizers has enhanced losses of nitrogen (N) and phosphorus (P). Monitoring in 2022 showed that N surplus and soil organic matter (SOM) deficiency in agricultural soils is widespread (Vries et al., 2022). The European Union (EU) depends mainly on imported fertilizers, the price of which is influenced by distribution costs and overall demand, the latter increasing with population growth. Price and availability of P rock can be influenced by geopolitical risks or natural disasters (Cordell et al., 2015; Smol, 2021), as the production of mineral P fertilizers depends on the limited resources of P rock, which is mainly controlled by five countries (Driver et al., 1999). To ensure food security, the production and use of fertilizers needs to become more sustainable. Therefore, nutrient recycling should be encouraged through recovering nutrients in the form of secondary resources.

To minimize negative impacts on the environment, the EU is working on achieving a circular economy by 2050, creating a sustainable food system and protecting biodiversity (European Commission, 2019). The EU has adopted several measures to protect the soil and slow down climate change. Among other benefits of the EU Green Deal, it aspires for healthy soil, biodiversity, and healthy and affordable food. Developing sustainable and organic agriculture, and drastically reducing the use of chemical pesticides and mineral fertilizers in all types of agriculture, is suggested to achieve these goals (European Commission, 2019). Bio-waste recycling provides an opportunity to return organic matter and nutrients to the soil, improving soil health, fertility and structure (Sánchez et al., 2017).

Based on the EU Green Deal, a roadmap for the Estonian circular bioeconomy is currently being prepared, with the purpose to define the main directions and goals until 2035. One of the goals is to valorize underutilized biodegradable waste, residues and by-products as a fertilizer/soil amendment to replace mineral fertilizers and keep nutrients in circulation. Estonia is sparsely populated, resulting in relatively small volumes of bio-waste in most regions. Long distance transport of these wastes to larger treatment centres is not economically feasible. Hence, one challenge of developing circular bioeconomy in Estonia would be promoting local facilities for bio-waste treatment (Ministry of Rural Affairs, 2023). Proximity composting is a suitable solution for diverse bio-waste types enabling reduced transportation costs of bio-waste and resultant product.

Most common input materials for composting are food waste, yard waste, manure, and sewage sludge (Lim et al., 2016), while fish waste is less common (Harrington et al., 2005; Radziemska et al., 2019). Fish waste is also a suitable input material for composting, but due to odour emissions and rapid deterioration, it has received little attention. Fish waste also qualifies as animal by-product (ABPR) and has more strict requirements for handling (Commission Regulation (EU) No 142/2011). Wastes from fish processing industries and aquatic by-catch are sources of fish waste. By-catch is discarded fish, including fish of no-target species, unsuitable size, damaged, or of no economic value (Harrington et al., 2005). Using the example of Lake Võrstjärv, the second largest lake in Estonia, the average amount of by-catch per 1 kg of commercial fish harvested in 2015-2019 was 1.3 kg (Silm et al., 2020). Thus, on average, 56% of the total catch is discarded as by-catch. However, by-catch is gathered unevenly and in quantities that are too small for biogas or fish meal production. Therefore, rather than being revalued, by-catch is often discarded back into the aquatic environment, causing aquatic pollution (Silm et al., 2020). On the other hand, if by-catch is collected regionally, this quantity is sufficient for proximity composting.

This study focuses on valorization of fish waste, an underutilized type of bio-waste, through proximity composting, examining the feasibility of the composting process and providing respective guidelines. In addition, the composts comprised of different bio-waste types are evaluated based on non-routine quality indicators like content and properties of phosphorus forms and humic substances.

# 2. LITERATURE REVIEW

## 2.1. The need for bio-waste management in circular economy

The United Nations adopted Sustainable Development Goals (SDG) in 2015 to protect the planet and ensure the prosperity of future generations (Lal et al., 2021; United Nations, 2016). The recycling of organic matter is indicated throughout these objectives. Across the EU, an estimated 118 to 138 million tonnes of bio-waste is generated, only 60 million tonnes of which is recycled through composting and anaerobic digestion (Severin et al., 2021). In 2021, 100,431 tonnes of bio-waste in mixed household waste and 45,028 tonnes of separately collected bio-waste were generated in Estonia, of which less than 30% is recycled (Taraskin, 2022). Unsegregated bio-waste is incinerated or disposed of in landfills, where it generates greenhouse gases (GHG), such as methane (Nanda & Berruti, 2021). According to SDG 12, organic wastes must be recycled more effectively, thus increasing our independence from mineral fertilizers and promoting development of sustainable agriculture (Lal et al., 2021). Recycling organic wastes helps to reduce the linear disposal to landfills and close the cycle of nutrients (Figure 1). The conventional alternative to landfilling bio-waste is composting, which helps to achieve the goal of SDG 13 and considerably reduces GHG emissions (Nordahl et al., 2020; United Nations, 2016). Furthermore, if organic fertilizers, such as composts, are directly applied to the soil or growing media, then organic carbon ($C_{org}$) in organic waste materials can be accounted as a net contribution to offset $CO_2$ emissions (Confalonieri, 2022).

Depending on the input material, waste processing technology can be selected to produce nutrient rich organic fertilizers or soil improvers. the EU Fertilizing Products Regulation sets limits for primary nutrient concentrations, organic C content and biological stability in solid organic fertilizers and soil improvers (Regulation (EU) 2019/1009). Nutrient rich composts hold high potential as organic fertilizer and offer the possibility to reduce usage of synthetic fertilizers (European Commission, 2019).

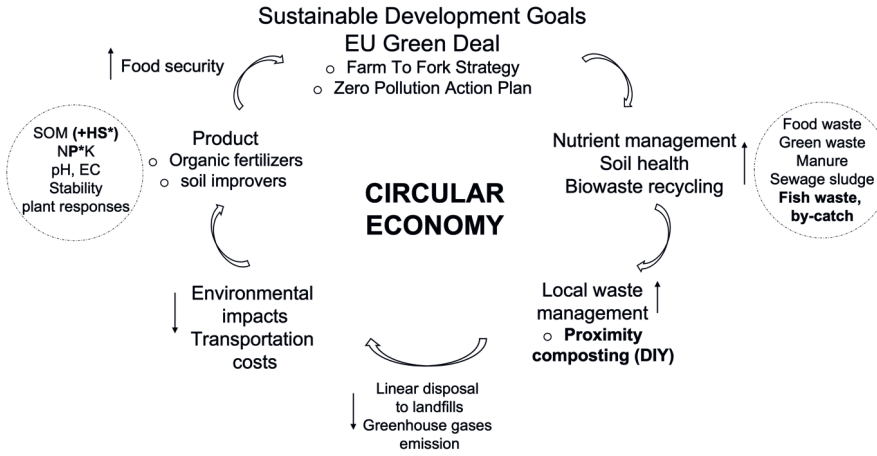


**Figure 1.** Scheme of the interdisciplinary nature of composting in the circular economy, adjusted from Korhonen et al., (2018). Arrows ↑↓ indicate, respectively, increasing or decreasing effects due to circular economy. Abbreviation DIY (do it yourself) refers to employment possibilities. Left circle includes compost quality parameters: SOM (soil organic matter, including humic substances (HS)); NPK is nutrient composition of nitrogen, phosphorus, and potassium; EC is electrical conductivity. Current thesis contributed to topics marked in bold. Asterisk * indicates quality parameters researched during present thesis.

Sustainable treatment of bio-waste requires separated collection from residual waste at the source (Brusselaers & Van Der Linden, 2020). Directive (EU) 2018/851 of the European Parliament and of the Council of 30 May 2018, amending Directive 2008/98/EC, stipulates an obligation at the EU level to implement separate bio-waste collection as of 1 Jan 2024 (Directive (EU) 2018/851). The quality of compost is highest in countries where municipal bio-waste is separately collected, and the input material is regulated (Brusselaers & Van Der Linden, 2020). In addition, the principle of proximity implies that, to reduce transportation costs and environmental impacts, wastes should be managed as near as possible to its generation source. This concept is accompanied by increased awareness of bio-waste recycling and encourages solving problems at the local level, while also creating new job opportunities. This outcome contributes to achievement of SDG 8 „decent work and economic growth“ (United Nations, 2016).

## 2.2. Composting process

Composting is biological decomposition of organic material under aerobic conditions. During composting, organic waste is converted

to stable material, and phytotoxicity, pathogens, and weed seeds are eliminated (Rynk et al., 1992; Sayara et al., 2020). Composts vary in quality depending on several factors, primarily the type of input materials and the duration of composting. A large variety of input materials can be composted alone or blended with materials that add structure and porosity, adjust carbon/nitrogen ratios (C/N), and add or adsorb liquids (Diaz et al., 2011).

Composting can be implemented at different scales and is therefore suitable for recycling bio-waste at local levels. However, attention must be paid to compost quality. Immature compost still containing easily degradable organic compounds can produce odours, decrease soil N and $O_2$ concentrations, generate compounds that can harm seedlings or sensitive plants, and inhibit plant growth (Emino & Warman, 2004; Harrison, 2008; Rynk et al., 1992). To transform bio-waste into a valuable product that can be used to improve plant growth and soil fertility, the composting process must be monitored.

Beneficial properties of compost can be enhanced by optimizing the composting process. To accelerate composting, biodegradable waste is shredded and moistened, if necessary (Lanno et al., 2020). The main factors for efficient and high-quality composting are temperature, water content, and aeration rate. The initial C/N ratio, in a range of 25–30:1, is a key factor to determine composting kinetics (Zhou et al., 2022). C/N ratio lower than 10:1 or higher than 40:1 will inhibit the growth of microorganisms necessary for degradation and composting (Bachert et al., 2008). Additives, such as clay minerals, can help reduce odours, improve conditions for microorganisms, and create optimum environments for humus formation (Diver, 2004).

Composting is an exothermic process, where temperature increases over 55 °C within the first few days. On an industrial scale, time-temperature profiles are required by legislation or quality assurance schemes, with the main purposes to ensure the absence of pathogens and weed seeds (Regulation (EU) 2019/1009; Siebert & Auweele, 2014). Temperatures over 70 °C are always targeted if ABPR are treated to ensure the absence of harmful pathogens (Commission Regulation (EU) 142/2011).

High temperatures indicate activity of microorganisms, which is accompanied by high oxygen consumption. Therefore, it is important

to aerate the material undergoing composting, for example by mixing or with passive aeration systems. It is especially important to mix the compost if $CO_2$ concentration exceed 16% or if $O_2$ concentration is below 5%. Decomposition occurs most rapidly at a temperature range of 40–60 °C; therefore, turning is recommended at higher temperatures (Rynk et al., 1992) (Table 1).

**Table 1.** Parameters of the composting process (Bachert et al., 2008; Rynk et al., 1992; Siebert & Auweele, 2014)

| Parameter | Value | Comments |
|---|---|---|
| Environment | Aerobic | |
| Need for turning the compost | $CO_2$ >16% or $O_2$ <5%<br>Temperature <40 °C | Aeration by turning the compost |
| Optimal temperature | 55–60 °C | |
| Sanitisation | Feedstock <70 °C<br>Animal by-products (ABPR) > 70 °C | Reduction of weed seeds >60 °C<br>Reduction of pathogens >70 °C |
| Water content | 40–70% | Moisture <40% add water<br>Moisture >70% add dry bulking material |
| pH | 7 – 11 | |
| C/N ratio | 25–30:1 | Initial C/N ratio of input material |

Once biological activity decreases, temperatures begin to decline. The degradation phase is followed by the conversion phase, where components that are difficult to break down continue decomposing with lower intensity. When temperatures decrease to ambient levels, the maturation phase starts, and humus forms (Figure 2) (Bachert et al., 2008).

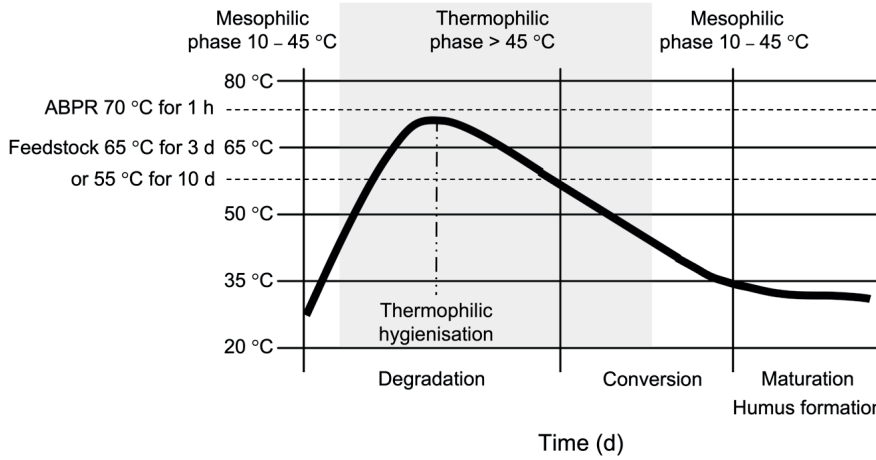


**Figure 2.** Temperature curve of the composting process adjusted from Bachert et al., (2008). Required temperatures for thermophilic hygienization of animal by-products (ABPR) and feedstock refer, respectively, to EU Commission Regulation on handling ABPR (Commission Regulation (EU) 142/2011) and Regulation on compost production (Parliament of Estonia, 2021).

## 2.3. Compost benefits

SDG 15 states that land and soil must be protected (Lal et al., 2021). Due to the changes in land use, agricultural practises, and climate, 45% of soils in Europe are low in organic matter content, which impairs soil productivity and crop yields and increases the risk for soil degradation (European Commission & European Environment Agency, 2012). Composts add organic material to the soil, improve soil health, and help to recover humus in degraded soils (Sánchez et al., 2017). Long-term use of compost enhances the aggregation of soil particles and improves soil fertility and C-sequestration, which may prevent erosion, eutrophication, and desertification. On the contrary, chemical fertilizers exhaust the soil in the long term (Hettiarachchi et al., 2020; Massah & Azadegan, 2016; Savci, 2012).

Composts have several benefits, and mature composts render soil more fertile by increasing plant available nutrients (N, P, potassium (K), calcium (Ca), magnesium (Mg)), and soil organic matter (SOM) (Barral et al., 2011; Khater, 2015; Milinković et al., 2019). Composts contain nutrients that are partially in organic form and are released slowly; therefore, fewer nutrients are leaching from soils compared to inorganic fertilizers, turning the compost into a long-term fertilizer (Barral et al., 2011).

The effects of compost on plant growth and soil properties depends on many factors, such as the type of compost and soil, the application rate, and target plants (Adani et al., 2007). For example, in clayey soils, compost can improve soil drainage and aeration by increasing porosity and aggregation; in sandy soils, compost improves water holding capacity and soil structure (Gagnon et al., 2001; Varghese et al., 2021). Compost application to soils decreases the risk of erosion and nutrient leaching (Bhattacharya et al., 2016) and affects soil pH and buffering capacity, which influences P uptake by plants (Ibrahim et al., 2022; Latifah et al., 2018). The quantity of compost applied to the soil can be calculated based on nutrient concentrations in compost and soil (mainly N and P). According to Estonian Water Act, up to 170 kg of N per year, or up to 25 kg of P per year, can be added to agricultural fields (Parliament of Estonia, 2019a).

Depending on nutrient concentrations, the EU fertilizer regulation divides composts into solid organic fertilizers or, more often, into soil improvers, due to insufficient NPK content (Regulation (EU) 2019/1009). Therefore, different composts might be effective for different purposes, depending on their primary nutrient value, but also SOM and HS contents, which help to improve physical and chemical properties of soil. HS concentrations in compost provide added value in addition to fertilization properties. HS are stable, long-lasting biomolecules that promote nutrient uptake, increase soil porosity, enhance nutrient preservation and water-holding capacity, and reduce the abundance of pathogens (Guo et al., 2019; Jindo et al., 2020; Mosa et al., 2020). Furthermore, HS can increase shoot biomass via hormonal effects on root elongation and plant development (Qin & Leskovar, 2020). HS can bind cations (Mg, Ca, Fe, but also Cd and Pb) and reduce negative effects of herbicides and toxic substances. These features are important for soil restoration processes needed to ensure safe food sources and sustainable agriculture (Gerke, 2018; Guo et al., 2019; Trevisan et al., 2010).

Compost application improves plant yields (Aydinsakir et al., 2009), but making general recommendations for the use of compost can be complicated, since they differ in nutrient concentrations and quality. The contents of nutrients, organic matter, and HS are affected by input materials and composting technology (Dores-Silva et al., 2018; Khater, 2015). For example, fish residues and fish compost show higher P release

capacities and are rich in calcium (Ca). They also behave like slow-release N fertilizers, minimizing N leaching to surface waters (Laos et al., 2000). Several composting trials have indicated that fish waste compost can increase crop yield and is a suitable fertilizer for agricultural use (Illera-Vives et al., 2017; López-Mosquera et al., 2011; Radziemska et al., 2019).

Although composts can be rich in total P, much of this P is unavailable to plants (Siebert & Auweele, 2014). For plant production, bioavailable P is needed for photosynthesis and respiration to promote root development and formation of seeds and fruits (Karthika et al., 2018; Uchida, 2000). Bioavailability is connected with solubility, since water soluble P forms are most bioavailable. The highest P solubility is estimated to be at around pH 6.5 (Penn & Camberato, 2019).

According to regulations, hygiene parameters for composts must meet the standards, and total nutrient concentrations must be reported (Regulation (EU) 2019/1009; Siebert & Gilbert, 2018). In Estonia, composting process and quality criteria are determined by regulation nr RT I, 18.12.2020, 23, „Requirements for production of compost from biodegradable waste", but compost stability, bioavailable forms of nutrients, and HS concentration are not regulated (Parliament of Estonia, 2021). Stability is an important parameter in the evaluation of composts and is often assessed according to regulation nr RT I, 06.08.2019, 7, "Requirements for the quality and usage for sewage sludge used in landscaping, recultivation and agriculture", which sets quality limits and requirements for use of sewage sludge. This regulation specifies that oxygen consumption must be less than 10 mg $O_2$/g of dry weight after a 96-hour measurement period (Parliament of Estonia, 2019b). Considering degradation of soils, in addition to fertilizing and general soil improvements, it is necessary to increase our understanding of HS, and comparing various composts will improve our knowledge of their value to end consumers and society.

# 3. AIMS AND HYPOTHESES OF THE STUDY

Through composting, organic wastes can be stabilized and turned into solid organic fertilizers and soil improvers. The evaluation of total nutrient concentration and meeting hygiene parameters of composts are required with the regulations (Regulation (EU) 2019/1009; Siebert & Gilbert, 2018). However, some beneficial properties of compost are not reported. To increase knowledge about the added value and properties of composts, the following aims and hypotheses were established.

The aims of the present study were to:

- recycle fish by-catch and fish waste on site and convert this resource into nutrient rich fertilizer/soil improver (**I**);
- determine the relative share and distribution of phosphorus (P) forms in fish waste compost and compare the results with composts from other organic input materials (**II**);
- determine the content and properties of HS in compost depending on input materials (manure, sewage sludge, fish waste, food waste, yard waste) (**III**).

Based on existing knowledge and previous research, the following hypotheses were established:

- proximity composting of fish waste is technologicaly feasible and has potential to support conversion to a local, circular economy (**I**)
- fish waste compost contains a high concentration of P, most of which is bioavailable to plants (**II**);
- the concentration of HS in compost depends directly on the input material (**III**).

# 4. MATERIALS AND METHODS

## 4.1. Labelling of composts

The composts presented in the published articles have been prepared within various projects. As a result, some composts have been described in several articles and identified by various labels. For clarity, composts are given new labels here, which will be used throughout this PhD thesis (Table 2). The origin, treatment, and specific analysis of composts are presented in Figure 3.

**Table 2.** Identifying labels of composts.

| Lable in present thesis | Target waste | Paper I | Paper II | Paper III |
|---|---|---|---|---|
| F1 | By-catch fish | F1 | FW1 | - |
| F2 | By-catch fish | F2 | FW2 | - |
| F3 | By-catch fish | F3 | FW3 | C1 |
| F4 | By-catch fish | F4 | - | - |
| F5 | By-catch fish | F5 | - | - |
| A1 | Horse manure | - | HM | C2 |
| A2 | Sewage sludge | - | SS | C3 |
| A3 | Green waste from municipal areas | - | GW | C4 |
| A4 | Kitchen bio-waste (ABPR chicken body parts) | - | - | C5 |
| A5 | Cafeteria and grocery store bio-waste | - | - | C6 |
| A6 | Kitchen bio-waste | - | - | C7 |

**Figure 3.** Overall scheme of PhD thesis.

### 4.2. Open windrow composting of fish waste (I)

Three batches of fish compost were produced from spring to autumn 2018. All composts contained wheat straw, produced in Taali, Pärnu County, Estonia, and fish waste from Lake Võrtsjärv, but they differed in pre-treatment and ratios of input material, additives, and composting time (Table 3). The target C/N ratio of input materials was 30:1, which was achieved by using a balanced ratio of input materials. Amendments, such as peat and mature compost, were added to compost F3. To test the suitability of lake sediment as a composting additive, two additional batches of fish compost were prepared in summer to autumn 2019. Input materials for F1, and further mixing to provide oxygen, were made using either a Backhus 16.30 windrow turner (Eggersmann, Germany) or an ST 300 windrow turner (COMPOST and WASTE technology, Austria). Input materials for F2–F5 were mixed using a BVL van Lengerich feed mixer V-Mix 13 2S plus (BVL Group, Germany).

To eliminate odours from fish decomposition, windrow F1 was covered with peat for 2 weeks, starting on day 7. To prevent the windrow covering with peat, for further experiments (F2–F5), a biofilter with negative pressure was used to eliminate odours. Detailed descriptions of compost preparation, monitoring, and biofilter construction are provided in article (**I**).

**Table 3.** Fish waste composting process conditions (includes data from article **I,** Table 1 and Table 2). The active period (d) indicates the duration in days that composts were in outdoor windrows. Quality requirements are specified according to Estonian bio-waste composting regulation nr RT I, 18.12.2020, 23 (Parliament of Estonia, 2021).

| Compost | Target waste | Amendments | Scale | Meets quality requirements | Active period (d) |
|---|---|---|---|---|---|
| F1 | By-catch fish, residues from the fishing industry | Straw | Industrial | Yes | 185 |
| F2 | | Straw | Industrial | Yes | 70 |
| F3 | | Straw, peat, mature compost | Industrial | Yes | 50 |
| F4 | | Shredded straw, lake sediment | Industrial | Yes | 135 |
| F5 | | Shredded straw, mature compost, lake sediment | Industrial | Yes | 135 |

### 4.3. Preparation of alternative composts

Alternative composts were selected for comparison with the nutrient and HS contents of fish waste composts. As with the fish waste composts, outdoor windrows were used to prepare horse manure, green waste, sewage sludge, and kitchen bio-waste composts. In addition, *H. illucens* uncomposted frass and vermicompost were selected (Table 4).

**Table 4.** Characterization of alternative composts. The active period (d) indicates the duration in days that composts were in outdoor windrows (**III,** Table 1). Quality requirements are specified according to Estonian bio-waste composting regulation nr RT I, 18.12.2020, 23 (Parliament of Estonia, 2021).

| Compost | Target waste | Amend-ments | Scale | Meets quality require-ments | Active period (d) | Composting Site Location |
|---|---|---|---|---|---|---|
| A1 | Horse manure | Straw, peat, mature compost | Industrial | Yes | 90 | 58°28'41.08" N 24°49'28.66" E |
| A2 | Sewage sludge | Straw | Industrial | Yes | 120 | 58°13'37.89" N 26°23'10.17" E |
| A3 | Green waste from municipal areas | None | Industrial | Yes | >180 | 59°28'14.63" N 24°54'43.97" E |
| A4 | Kitchen bio-waste (ABPR chicken body parts) | Shredded wood | Industrial | Yes | 130 | 59°27'36.08" N 25°5'17.18" E |
| A5 | Cafeteria and gro-cery store bio-waste | None | Pilot | No | 51 | 58°23'30.76" N 26°41'40.43" E |
| A6 | Kitchen bio-waste | Straw, paper, green waste, biochar | Pilot | No | 365 | 58°23'30.76" N 26°41'40.43" E |

A5 is uncomposted frass from *H. illucens* bio-waste treatment
A6 is bio-waste treatment with vermicomposting technology

## 4.4. Chemical analyses

### 4.4.1. Determination of compost stability and nutrient content

Cumulative oxygen consumption was assessed over four days (96 hours) from the prepared fish waste composts (**I**). Samples were collected from all composts to determine concentrations of potassium (K), calcium (Ca), magnesium (Mg), and phosphorus (P) according to the ammonium acetate–lactate (AL) method (Egner et al., 1960). Total C and N contents were analysed using a Variomax CSN elemental analyzer (**I, II**).

### 4.4.2. Phosphorus fractionation (II)

To compare P forms in fish composts (F1–F3) and other common types of compost (A1–A3), a modification of the sequential extraction method was applied. According to Rydin (2000), six operationally defined P forms were determined (Figure 4):

Labile P – dissolved and loosely bound P;
Fe-P – P bound to reducible metals (Fe, Mn);
Al-P – P bound to aluminum (Al);
Org-P – P bound to organic material and colloids, determined as difference between soluble reactive P and total P in NaOH extract; and
Ca-P – P bound to calcium (Ca);
SumP – calculated as sum of P fractions.

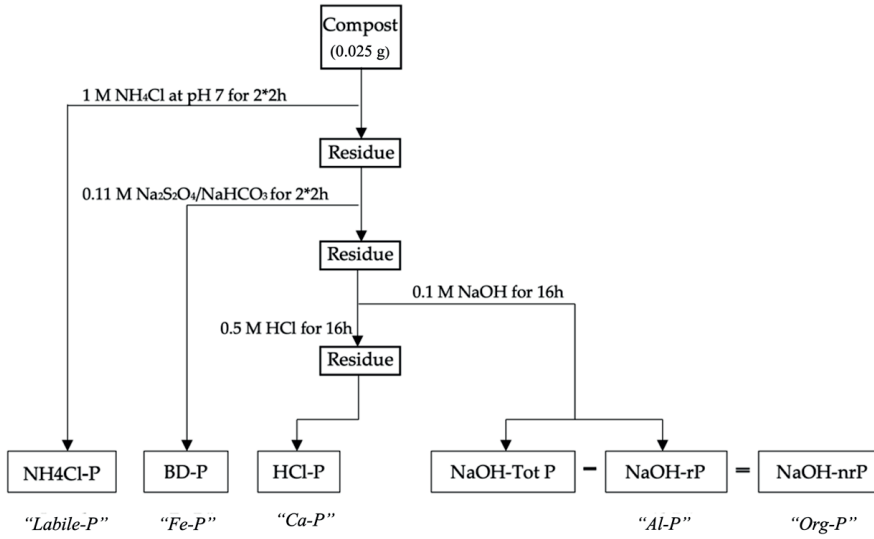


**Figure 4.** Phosphorus fractionation scheme described by Rydin (2000).

The P extraction method is described in detail in original article (**II**):

### 4.4.3. Characterization of humic substances (III)

Seven composts with different input materials were selected (F3 and A1–A6) to assess the content and form of to assess the content and form of humic acids (HA) and fulvic acids (FA). For this purpose, HA and FA solutions were prepared (**III)**. The absorbance of solubilized HA in a solution was measured using Ultraviolet-visible (UV-Vis) spectroscopy. The $E_4/E_6$ ratio was calculated from the UV-Vis absorption at 465 nm and 665 nm. To obtain HA vibrational spectra, Fourier-Transform Infrared Absorption (FTIR) Spectroscopy was used. Excitation Emission Matrix (EEM) Spectroscopy was used to obtain fluorescence spectra of HA and FA solutions. Total organic carbon (TOC) content in HA and FA solutions was measured using a CSN total carbon analyser, and the respective HA/FA ratio was calculated. Lipids were extracted from dried compost sample, air-dried and weighed. Then their proportion in the compost was calculated (**III**).

## 4.5 Statistical analyses

Statistical analyses were performed in R (R Core Team, 2020). The Shapiro–Wilk test was used to confirm normality (**II**). One-way ANOVA was used to study the effects of treatment on mean temperature (**I**); the effect of compost type on different P forms (**II**); and results of TOC and lipids (**III**). Three-way ANOVA was performed to examine the effects of treatment, ambient temperature, duration of composting, and the interactions between these factors, on composting temperature (**I**). A post-hoc Tukey HSD analysis was performed for pairwise comparisons using the package 'agricolae' (Mendiburu, 2015) (**I, II, III**).

# 5. RESULTS

## 5.1. Open windrow composting of fish waste (I)

All fish composting treatments reached the thermophilic phase in 1 to 2 days. Temperatures exceeded 70 °C, as required by EU Commission regulation EU 142/2011 on ABPR (Commission Regulation (EU) 142/2011). After the thermophilic phase, temperatures decreased, and ambient levels were reached (Table 5).

**Table 5.** Temperature characteristics: n.d. = no data; * = temperature fluctuation. In F2 treatment, temperature decreased to < 35 °C several times after day 6 and increased again after mechanical mixing with the windrow turner (**I,** Table 3).

| Batch Windrow | Thermophilic phase started > 45 °C | Number of days temp > 45 °C | Number of days temp > 70 °C | Peak temp °C | Peak temp achieved | Temp decreased < 35 °C |
|---|---|---|---|---|---|---|
| F1 | n.d | n.d | n.d | n.d | n.d | n.d |
| F2 | day 1 | 14 | 1 | 72.9 | day 1 | day 6* |
| F3 | day 2 | 21 | 7 | 76.4 | day 6 | day 23 |
| F4 | day 1 | 63 | 18 | 75.0 | day 7 | day 63 |
| F5 | day 2 | 69 | 21 | 78.6 | day 8 | day 73 |

The first composting experiment (F1) showed that a windrow turner is not suitable for initial homogenization of wheat straw and whole fish. The input materials did not mix well, and the whole fish were thrown from the windrow during mixing. Furthermore, the fish started to 'slow-cook' with the rising temperatures in deeper layers and started to dry or rot in outer layers of the windrow. As a result of covering the windrow with peat, the undesired odours from the fish decomposition processes were eliminated.

In further experiments, water was added to the input materials, and a feed mixer from a local farm was used for initial homogenization of the composting mixture. Further compost mixing with the windrow turner was successful. Initially, the biofilter caused temperature to decrease in the windrow when it was turned on for 15 min every 2 h. Adjustments during the experiment showed that the optimal biofilter work mode was twice per day for 15 min during the initial 4–5 days. The biofilter

provided sufficient $O_2$ supply to the windrow during these initial days, so the need for mechanical mixing was eliminated at this period.

In treatment F2, composting processes started immediately, and the thermophilic phase was reached at the end of day 1. Temperature fluctuations started after day 6, when temperatures fell below 35 °C (**I**, Figure 2). The compost pile was mixed with the windrow turner when temperatures decreased. As a result, temperatures temporarily increased. The thermophilic phase in treatment F2 reached the threshold for 14 days (Table 4).

After addition of peat and mature compost to F3, the compost became more homogeneous compared to F1 and F2. The thermophilic phase for this treatment was reached by day 2 (**I**, Figure 2), and temperatures remained high during the active composting period (23 days).

Mean windrow temperatures in F5 and F4 were not statistically different (**I**, Figure 3). For treatments F4 and F5, the thermophilic phase was reached by the end of day 1, and temperatures over 45 °C lasted longer than in previous treatments for 63 and 69 days, respectively (Table 5).

Three-way ANOVA analysis confirmed that composting temperature was affected by the treatment, ambient temperature, and composting duration ($P < 0.05$) (**I**, Table 4).

According to Estonian regulations, sewage sludge compost is considered stable if $O_2$ consumption over 96 hours is less than 10 mg $O_2$/g DW (Parliament of Estonia, 2019b). $O_2$ consumption over 96 hours remained slightly above 10 mg $O_2$/g DM in treatments F2 and F3 (**I**, Figure 4). Treatments F1, F4, and F5 windrows were very stable, with oxygen consumption below 5 mg $O_2$/g DW.

Nutrient concentrations from the mature composts are described in Table 6. Fish composts F1–F3 were selected and compared with other common types of compost. All composts were estimated to be mature and varied in nutrient concentration. N, P, and K are the primary nutrients for plant growth. N concentrations were highest in the A2 compost (3.74 %), followed by F5 (2.77 %) and F2 (2.75%). The A3 compost differed from others with a low N concentration (0.6 %). Concentrations of bioavailable P in fish waste composts (F1–F5) were 4–7 times higher than in A3 (1.15 g/kg). Potentially bioavailable P concentrations in fish

waste compost ranged from 4.88 g/kg in F3 to 9.19 g/kg in F5. The A2 and A3 composts had similar concentrations of K (5.52 g/kg). The A1 compost contained more K than other composts (25.51 g/kg).

**Table 6.** Fish waste compost (F1–F5), horse manure compost (A1), sewage sludge compost (A2), and green waste compost (A3) nutrient concentrations (determined with AL. method), stability per dry weight (DW). Different letters indicate statistically different groups according to Tukey HSD test ($\alpha = 0.05$). Superscript letter a indicates the highest concentration, while superscript letter d indicates the lowest concentration (table includes data from article **I,** Table 5 and Table 6; article **II,** Table 2).

| Compost | C | N | C/N | P | K | Ca | Mg | Stability | DW |
|---|---|---|---|---|---|---|---|---|---|
| | % | | ratio | AL meth. g/kg | | | | mg $O_2$/g DW | % |
| F1 | 30.22 | 2.43 | 12.44 | 6.39$^{b}$ | 7.91$^{cd}$ | 44.71$^{ab}$ | 7.03$^{a}$ | 3.0 | 53.8 |
| F2 | 33.04 | 2.75 | 12.01 | 5.93$^{bc}$ | 13.29$^{bc}$ | 46.26$^{ab}$ | 6.64$^{a}$ | 11.2 | 47.94 |
| F3 | 35.66 | 2.73 | 13.06 | 4.88$^{c}$ | 16.76$^{b}$ | 31.66$^{ab}$ | 6.81$^{a}$ | 10.4 | 34.79 |
| F4 | 27.92 | 2.77 | 10.08 | 8.02$^{a}$ | 6.68$^{cd}$ | 26.80$^{ab}$ | 2.91$^{c}$ | 2.8 | 39.91 |
| F5 | 27.64 | 2.74 | 10.09 | 9.19$^{a}$ | 10.31$^{cd}$ | 29.60$^{ab}$ | 3.01$^{c}$ | 3.0 | 32.75 |
| A1 | 30.99 | 2.47 | 12.55 | 1.36$^{d}$ | 25.51$^{a}$ | 29.86$^{ab}$ | 6.49$^{a}$ | 3.2 | 37.83 |
| A2 | 27.02 | 3.74 | 7.22 | 1.18$^{d}$ | 5.52$^{d}$ | 11.70$^{b}$ | 5.18$^{b}$ | 6.7 | 44.16 |
| A3 | 13.35 | 0.6 | 22.25 | 1.15$^{d}$ | 5.52$^{d}$ | 66.82$^{a}$ | 2.24$^{c}$ | 0.9 | 41.78 |

## 5.2. Phosphorus forms in composts (II)

P fractionation results showed that SumP in composts (Figure 5; **II**, Figure 2) ranged from 2.73 g P/kg dry weight (DW) in the green waste compost (A3) to 16.27 g P/kg DW in sewage sludge compost (A2) (**II**, Table 3). Fish waste composts (F1-F3) contained statistically similar SumP to horse manure (A1) and sewage sludge (A2) composts.

In addition to SumP, the composts varied in concentrations of P forms. Labile-P, the most bioavailable P form, was highest in fish composts, ranging from 6.90 g P/kg DW (F3) to 7.75 g P/kg DW (F2). The lowest concentration of labile-P was in the A3 compost (0.92 g P/kg DW).

While the A2 compost had the highest SumP, its concentration of labile-P was statistically similar to the A3 compost. In A2, most of the P was in Fe-P and Al-P form. In other composts, Fe-P and Al-P did not exceed 0.90 P/kg DW and 0.16 g P/kg DW, respectively.

The highest concentration of Ca-P was in fish composts (from 2.26 g P/kg DW in F3 to 4.27 g P/kg DW in F2). The A3 compost contained a considerable amount of Ca-P (1.57 g P/kg DW). The lowest concentration of Ca-P was observed in A1 (0.16 g P/kg DW).

Org-P concentration was similar in F2, F3, A1, and A2 composts, ranging from 0.54 g P/kg DW (A2) to 0.59 g P/kg DW (F3).

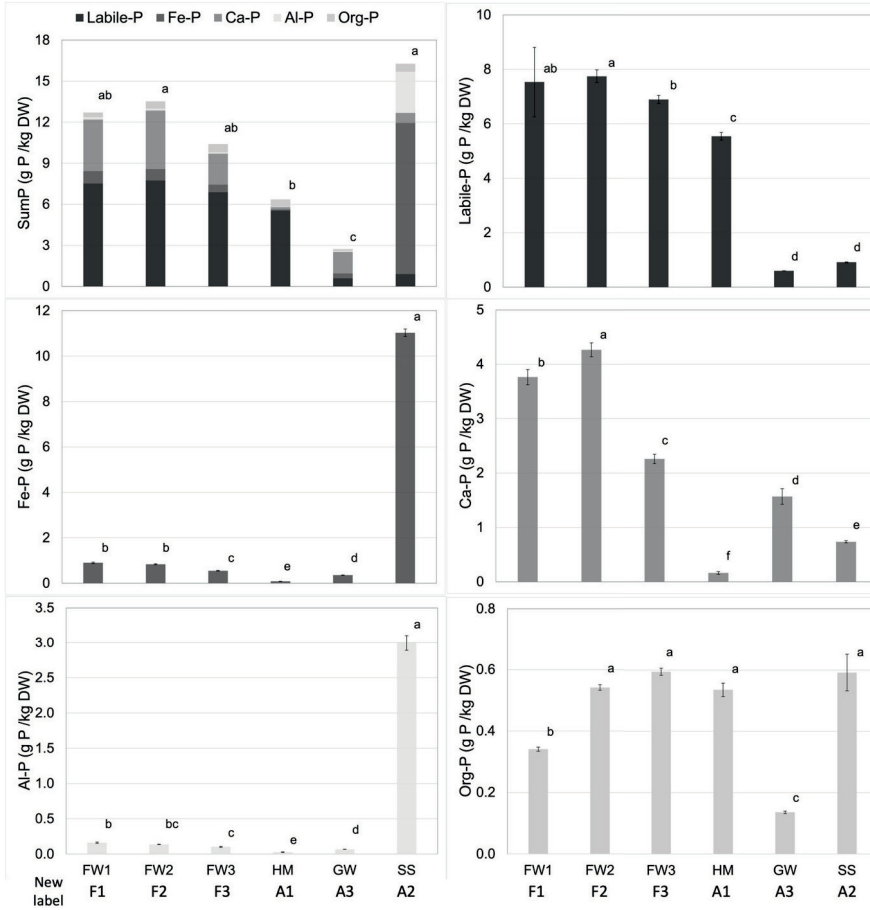


**Figure 5.** Concentrations of different phosphorus (P) forms (g P/kg DW). Labile-P is water soluble and loosely bound P; Fe-P is iron and manganese bound P; Ca-P is calcium bound P; Al-P is aluminum bound P; and Org-P is P bound to organic material. Different letters indicate statistical differences between groups, according to the Tukey HSD test ($\alpha = 0.05$). Labels indicate fish waste composts (F1, F2, and F3), horse manure compost (A1), sewage sludge compost (A2) and green waste compost (A3).

Composts also differed in relative share of P forms. The proportion of labile-P was highest in the A1 compost, accounting for 87% of SumP (**II**, Figure 3). While fish waste composts contained highest concentrations of labile-P per compost dry weight, the relative share of labile-P ranged 57–66% of SumP in composts F1–F3. The A2 compost, with the highest SumP concentration, contained the lowest proportion of labile-P (6%).

The proportions of Fe-P and Al-P remained relatively low in other composts (Fe-P < 13% and Al-P < 3%), but they constituted the majority in sewage sludge (A2) compost (Fe-P 68% and Al-P 18%). The proportion of Ca-P was highest in A3 (57%), followed by fish composts (up to 32%). In the A2 and A1 composts, Ca-P did not exceed 5%. The proportion of Org-P remained below 10% in every compost.

### 5.3. Properties of humic substances (III)

During compost maturation, the concentration of HA increases, and FA decreases (Raj & Antil, 2011). Therefore, the higher HA/FA corresponds to increased humification rates of organic material (**III,** Table 3). The highest HA/FA ratio was measured in treatments A1 (7.88) and A3 (7.82), whereas the lowest HA/FA ratio was observed in treatments A4 (1.42), A5 (1.46) and A6 (1.60). Treatments A5 and A4 had the highest concentrations of lipids (2.1% and 4.2%, respectively).

The spectral ratio ($E_4/E_6$) depends on the source material, shows overall aromaticity and condensation of material, and can characterize the size of molecules (**III,** Table 3) (Y. Chen et al., 1977). $E_4/E_6$ varied from 6.40 in A5, indicating more advanced polymerization of organic material, to 8.59 in A6.

UV-Vis spectra values for HA decreased monotonically with increasing wavelength (**III,** Figure 1). Absorption peaks between 255 and 290 nm were detected for F3, A1 and A2, while these shoulders of spectra were smaller for A3, A5 and A6. Compost A4 did not exhibit a shoulder of spectra. These peaks are typical for aromatic or unsaturated compounds, such as quinones and ketones (Klavins & Purmalis, 2013).

Analyzed compost samples had similar FTIR spectra absorption patterns at 3600–3000 $cm^{-1}$, representing -OH, and methyl groups in aliphatic compounds that were detected at 2920 $cm^{-1}$ (Verillo et al., 2021) (**III,** Figure 1). The spectra showed the absorption of C=O bonds in carboxyl groups, aldehydes, ketones, and esters, with higher rates in samples A2, A3, A4, and A6 (at 1700 $cm^{-1}$). Compost A6 had significantly, and A1 had moderately lower, signals in the region at 1637–1610 $cm^{-1}$. Treatments A4 and A6 differed from other samples, while A4 had low absorptions of C-N and N-H bonds in amides, and A6 had none. These treatments also had visible differences on the region at 1470–1370 $cm^{-1}$, representing carbonyl acids and phenols, where the presence of those bands was low. Carboxylic groups and ethers were present in HA samples from all treatments, with slightly higher amounts for F3 and A3. The presence of C-O in carboxyl groups and ethers is common and was similar for all samples. The region at 1070–1020 $cm^{-1}$, describing C-O bonds in polysaccharides, was noticeably lower in A1 and A4 samples. The presence of detected structural features describes the condensation and conjugation of organic material during composting processes.

EEM fluorescence spectra for FA and HA are shown in Figure 6 (**III**, Figure 3) and Figure 7 (**III**, Figure 4). All analyzed samples revealed a peak associated with polysaccharides and were related to the FA visible wavelength area (Ex/Em 340–350/440–450 nm). A very slight peak was present at Ex/Em 420–450/520–550 nm, representing condensed aromatic structures (Valencia et al., 2013; Wei et al., 2014).

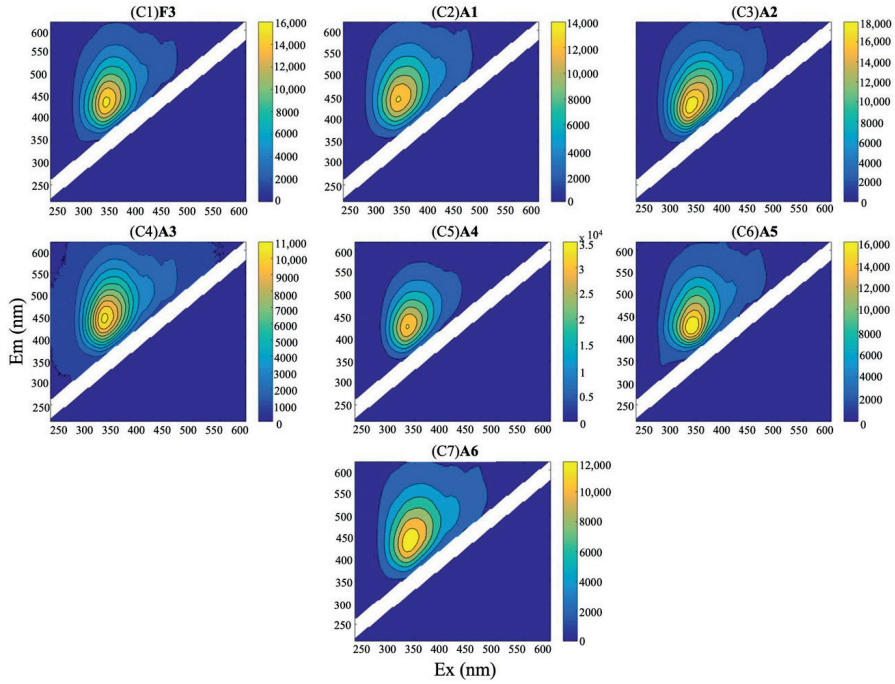


**Figure 6.** The EEM spectra of fulvic acids (FA) in different treatments. Labels indicate fish waste compost (F3); horse manure compost (A1), sewage sludge compost (A2), green waste compost (A3); kitchen bio-waste compost (A4), *H. illucens* frass (A5) and vermicompost (A6).

EEM matrix patterns of HA have different compositions and dominant peaks (Figure 7). Common peaks vary only by dominant peak and relative height. HA-like substances form during humification of composting organic matter and are associated with Ex/Em 350–380/470–510 nm (da Silva et al., 2020). This peak dominated detected compounds in samples F3, A1, and A4, and was the second largest peak in samples A3 and A5. Similarly, to FA, the EEM matrix for HA has Ex/Em 430/520 nm peaks, associated with degradation and condensation of organic material. Samples A3, A4, and A6 dominated in Ex/Em 460–470/540–560 nm, probably indicating lignin-like structures from structural amendments (shredded wood, straw, paper). A5 differed from other samples, with the core of HA peaking at Ex/Em 370–410/610–630 nm. The origin of these compounds could be fresh plants and fruits added to the treatment and characterized by low degradation rates and chlorophyll (Richard et al., 2009).

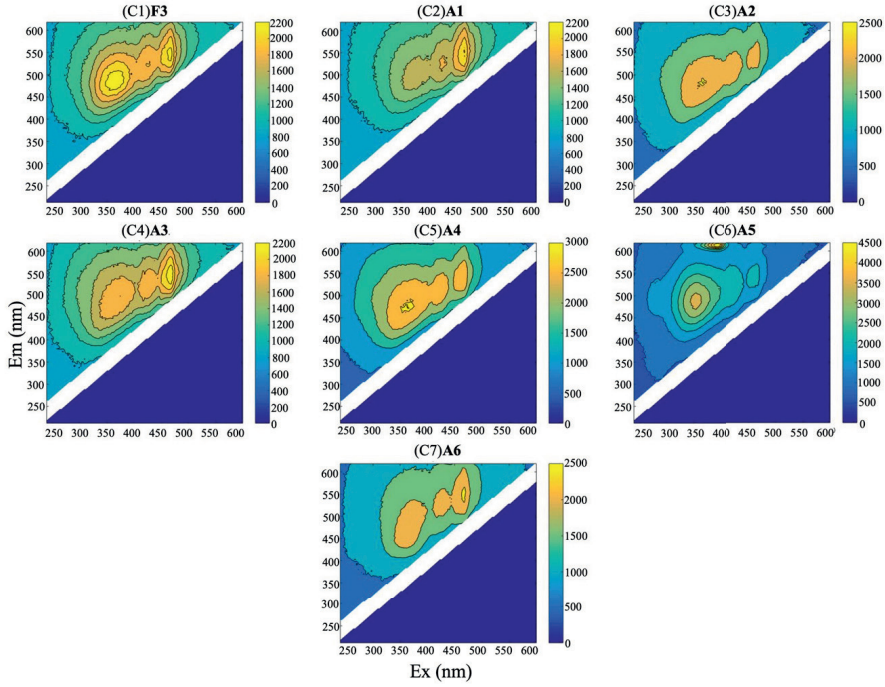


**Figure 7.** The EEM spectra of humic acids (HA) of different treatments. Labels indicate fish waste compost (F3); horse manure compost (A1), sewage sludge compost (A2), green waste compost (A3); kitchen bio-waste compost (A4), *H. illucens* frass (A5) and vermicompost (A6).

Figure 8 presents TOC in the summarized HA and FA pool, compared to P concentration, all expressed per compost DW (articles **II** and **III**). The highest concentrations of TOC were in horse manure compost A1 (27.0 ± 0.2) and sewage sludge compost A3 (25.7 ± 0.2). At the same time, A1 also had high concentrations of labile-P (5.55 g P/kg DW), while labile-P concentration in A2 was relatively low (0.92 g P/kg DW). The fish waste compost F3 had the lowest concentration of TOC in this comparison (12 ± 0.2%) but had the highest concentration of labile-P (6.90 g P/kg DW) (**III,** Table 3).

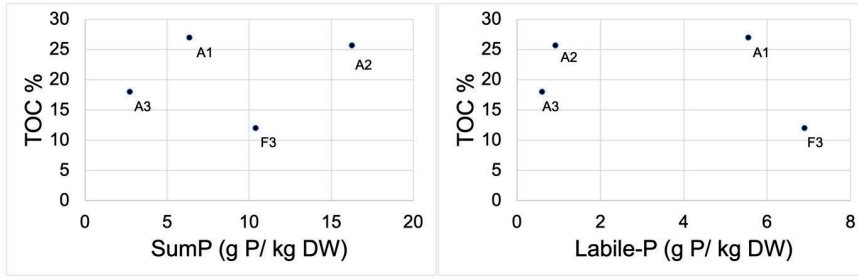


**Figure 8.** Comparative evaluation of total organic carbon (TOC) contained in summarized HA and FA pool (value given as percentage of compost DW) with SumP and Labile-P (g P/kg DW) concentrations in composts. Labels indicate fish waste compost (F3); horse manure compost (A1), sewage sludge compost (A2), green waste compost (A3).

# 6. DISCUSSION

## 6.1. Open windrow composting (I)

Open windrow composting is a relatively simple and feasible way to recycle various types and volumes of bio-wastes (Bachert et al., 2008). To minimize transportation costs, offer jobs locally, and prevent fresh fish from spoiling, one focus of the current study was processing fish waste by open windrow composting near fishing sites. In the framework of a circular economy, composting fish by-catch interrupts the unidirectional flow of P and other nutrients to surface waters, instead returning these valuable nutrients to the soil to improve soil fertility and plant growth (Ahuja et al., 2020; European Commission, 2020b).

Various bulking agents can be used in composting fish, such as straw, pine bark, sawdust, seaweed, and peat (Illera-Vives et al., 2017; Radziemska et al., 2019). In the present study, wheat straw was chosen as a bulking material due to its airy structure and wide availability in Estonia. Since wheat straw is dry, moisture in the composting mixture must be well balanced. In the first treatments (F1, F2 and F3), unshredded straw did not completely degrade during composting, and straw pieces were recognized as unchanged in the final compost. Straw contains a large fraction of cellulose (29–35%), hemicellulose (26–32%), and lignin (16–21%) (Kapoor et al., 2016), resulting in relatively slow decomposition (Eklind et al., 2007). Composting experiments F4 and F5 showed that composting processes accelerated if wheat straw was shredded before mixing the input materials. Shredded straw is easier to mix, and it decomposes and releases its C to the microorganisms more easily, than unshredded straw (Rynk et al., 1992).

Regulations require that, when composting animal by-product (ABPR), temperatures must exceed 70 °C for at least 1 hour (Regulation (EU) 142/2011). In all fish waste composting treatments, the thermophilic phase was reached within 1 to 2 days, and the required temperature was achieved. Therefore, outdoor windrow composting of fish waste is feasible in the spring to autumn period, which coincides with the by-catch collection period. During low temperatures in winter, composting processes slow down or cease, shortening the suitable time for outdoor composting in boreal regions (Rynk et al., 1992).

The first composting experiment (F1) confirmed that, during fish decomposition, unpleasant odour disturbances occur. As a mitigation measure, the F1 windrow was covered with peat. After 2 weeks, the peat was removed, and the odour problem was resolved. During experiment F2, a biofilter with negative pressure was used to solve the odour problem. Using the biofilter twice per day was effective for smaller-scale windrows (12–16 m long and 1.5 m wide) by supplying the composting process with sufficient oxygen and by eliminating negative odours. Therefore, forced airflow into the pile also reduced the need for mechanical mixing during the first days. For larger-scale industrial windrows, such aeration frequency may not be sufficient, and oxygen depletion may occur within 20–60 minutes (Epstein, 2011).

Experiments F1 and F2 were initiated with minimum possible input materials, using only fish waste and unshredded straw. During decomposition, high temperatures are related to high microbial activity (Alves et al., 2019; Jurado et al., 2015). Therefore, temperature fluctuations in treatment F2 indicated unstable microbial activity during composting, resulting in more undecomposed organic matter. In experiment F3, peat and mature compost were added to increase microbiological activity and mixing of input materials. As a result, no large temperature fluctuations were observed during the thermophilic composting phase.

The fish waste composting experiments showed that the thermophilic phase in windrows were sustained for longer periods of time when additives, such as peat, mature composts, and clay sediments, were used, also suggesting increased decomposition.

Treatments F4 and F5 exhibited the most active composting, which may reflect the addition of shredded straw and clay minerals from from lake sediments. Compost constituents were bound more suitably, making the mixture more homogeneous and providing better conditions for decomposition. The addition of clay minerals can increase water retention capacity and binds organic material during composting (Chen et al., 2018). Therefore, adding clay minerals assures more favourable conditions for microbial activity, resulting in higher temperatures and a longer duration of the thermophilic phase. All fish waste composts were stable and suitable for use.

## 6.2. Phosphorus forms in composts (II)

Input materials affect nutrient concentrations, but also the bioavailability of P (Sharpley & Moyer, 2000; Wei et al., 2015). Fish composts (F1–F3), horse manure (A1), sewage sludge (A2), and green waste (A3) were studied to identify the P forms and evaluate their dependence on input materials.

In the experiment, separate P forms and their summarized pool (SumP) were calculated. SumP differs from total P concentration by also considering residual P (Rydin & Otabbong, 1997). For fertilization, residual P is a stable form that does not add to the pool of bioavailable P (Blake et al., 2003; Carter & Gregorich, 2007).

All selected composts varied in P concentration. The high SumP concentration in A2 is presumably related to sewage sludge origin from a small-scale wastewater treatment plants (WWTP), where $Fe(SO_4)_3$ and $Al_2(SO_4)_3$ is used for chemical precipitation of P in wastewater. Fish waste also has high P content, but the P bioavailability in A2 and F1–F3 composts were different.

P concentration in composts is influenced by the relative proportion of bulking material and composition of the waste. Total P concentration in fish and fish waste ranges from 10–35 g P/kg DW, and in fish bones above 120 g P/kg DW (Ahuja et al., 2020; Dantas & Attayde, 2007; Ramadhani et al., 2018). Fish waste is a good source of P for plant growth (Ahuja et al., 2020; Radziemska et al., 2019). In the present study, over 50% of P in all F1–F3 composts was in a labile, readily bioavailable form.

There was a relatively high proportion of Ca-P in fish composts, which is regarded as a stable P form (Naidu et al., 2008; Yan et al., 2020). This Ca-P is most likely due to high concentrations of hydroxyapatite in fish bones and scales. Although fish waste composts contained the highest concentration of labile-P per DW, the relative proportion of labile-P was highest in the A1 compost. As F1–F3 and A1 composts had high ratios of readily bioavailable P, they have a high potential to partly replace mineral P fertilizers, when rapid P effect is needed.

The way compost is used depends on the source material. One advantage of composts compared to mineral fertilizers is the long-term fertilizing

effect, as plant nutrients are released slowly from organic matter decomposition. However, potential risks of soil saturation and nutrient leaching must be considered, especially near sensitive water bodies (Hurley et al., 2017). Water soluble nutrients are bioavailable for plants, but if composts are overused, these nutrients could easily leach from the soil. Plowing reduces runoff and water-soluble nutrient losses; therefore, it is recommended to use compost before spring plowing (Wortmann & Walters, 2007). In this way, plant nutrients are immediately used, and after the active growth period, compost functions as a soil improver. Knowledge regarding the distribution of P forms in composts could be valuable when evaluating composts as sources of P fertilizers (Sharpley & Moyer, 2000; Yan et al., 2020).

One alternative to reduce P leaching from soils and reducing P pollution in water bodies is co-composting wastes with higher proportions of bioavailable P together with low bioavailable P wastes (Wei et al., 2015). Therefore, co-composting fish waste and green wastes might be beneficial. Green waste, which is low in SumP and labile-P, enhances organic matter degradation, humification, and nutrient content when co-composted with other organic materials (Zhang & Sun, 2017).

In the present study, the A2 compost contained the highest overall P concentration per DW, in accordance with previous studies, where sewage sludge totP concentration was over 30 g P/kg DW; after mixing with soil, only 1–2% of the P was in a labile form (Rydin & Otabbong, 1997). Most of the P in A2 was bound to Fe and Al compounds. Nevertheless, depending on environmental conditions, P forms may change over time, affecting its availability for plants (Naidu et al., 2008; Yan et al., 2020). Related to the dynamic equilibrium between P pools, P is continuously transformed from other fractions into the bioavailable form (Naidu et al., 2008). It has been suggested that, when labile-P pools in soil are depleted, P bound to Al and Fe may become mobilized (Saleque et al., 2004). Al-P is considered a potentially bioavailable form, and, depending on soil pH, Al-P can be more bioavailable than Fe-P or Ca-P (Syers et al., 2008; Yan et al., 2020). Although Fe-P is a highly stable P form in oxic conditions, both Fe and Fe-P can dissolve when anaerobic soil conditions occur or when lower pH conditions catalyze Fe reduction (Syers et al., 2008; Yan et al., 2020). As 86% of the P in A2 was in fixed form (18% Al-P and 68% Fe-P), application of biological, rather than chemical, P removal in WWTPs could be considered when sewage

sludge is used as fertilizer. However, sub-zero winter temperatures in colder climates can inhibit biological P removal.

Limits for using A2 composts are subject to national wastewater regulations and may exceed limits for heavy metals or other safety and quality indicators (Council Directive 86/278/EEC). Previous studies assessed the potential of sewage sludge for agricultural use, indicating that A2 compost may represent an alternative to chemical fertilizers for crops of moderate nutrient demand (Eid et al., 2020; Elsalam et al., 2021; Tomócsik et al., 2016).

The type of organic acids produced during composting represents a potentially important attribute that can enhance phosphate rock solubility (Lichtfouse, 2010). Combining organic and mineral fertilizers may be beneficial because composts vary in nutrient concentration. This type of integration improves P solubilization, minimizes P precipitation in soil, and increases P availability for crops (Meena et al., 2018).

After compost application, P can become almost completely plant-available within three vegetation periods (Amlinger et al. 2006). Therefore, total P contents of composts can be calculated as a substitute for mineral P fertilization (Lichtfouse, 2010). Our research did not focus on the long-term release of P from the studied composts in the soil, but, depending on the compost, most P may be strongly bound, which can reduce bioavailability.

In addition to nutrients, composts also contain HS, which can interact with metal ions, oxides, hydroxides, and mineral and organic compounds, thus also affecting P availability (Albers et al., 2008). Humic-Fe complexes can decrease the formation of $FePO_4$ and increased the solubility of P in soil (Sundman et al., 2016). Furthermore, HS treatment can make P more available in the soil solution and stimulate root PM-ATPase, thereby increasing P absorption and levels in plant tissue (Jindo et al., 2020).

### 6.3. Humic substances in composts (III)

Various input materials contain different basic components that form humic substances (Dores-Silva et al., 2018; Guo et al., 2019). During composting, the concentration of humus-like substances and the degree

of humification of hydrophilic components increases, and the structure of the substance becomes more stable (Dores-Silva et al., 2018). To evaluate HS concentrations and forms, seven composts with different input materials were studied (F3, A1–A6).

During composting, the concentration of FA decreases, and HA increases. Compost is considered mature if the HA/FA ratio is higher than 1 (Ben Mbarek et al., 2019; Guo et al., 2019; Raj & Antil, 2011; Silva et al., 2009). The type of organic material affects the HA/FA ratio. Lowest HA/FA were measured in treatments A4, A5, and A6, which contained a high quantity of food waste. Sugars, such as polysaccharides, break down easily and are first to form FA and then HA. Highest HA/FA ratios were in treatments A1 and A3, which were horse manure and green waste composts, respectively. Because of fermentation in the animal gut, horse manure contains a high proportion of lignin (hay, grass), aromatic compounds, and older material. Green waste also contains high proportions of lignin, cellulose, and hemi-cellulose from plant remains and branches. Lignin and aromatic compound remnants could constitute the core of initial HA, and less FA is formed subsequently.

In addition to source materials, the content of HS depends on the amounts of nutrients (e.g., C and N) and the presence of microorganisms. As shown previously, chemical structures of HS are similar in compost treatments (Dores-Silva et al., 2018; Sutton & Sposito, 2005). UV-vis spectra of HA in the current work were similar to those described by Zilong&Yu (2021) as hydrophilic components decreased monotonically along the wavelength scale. The observed spectra were typical of aromatic or unsaturated compounds, such as conjugation of quinones and ketones. Although the composts varied in quality, HA was present in all treatments. HA formation rates tend to increase in the final stages of composting due to microbes that degrade and convert recalcitrant lignocellulosic biomass into a humus-stable form (Abdellah et al., 2022).

All compost treatments in the present study contained HS, including HA and FA. All treatments presented a FA peak for polysaccharides, but they differed relative to the HA peak, showing that input materials directly influence the core structure of HA in mature compost. Treatments F3, A1, and A4, which contained different ABPR (e.g., fish waste, horse manure, chicken body parts), had a dominant HA peak reflecting humic acid-like substances formed during humification. ABPR is rich in

nutrients and supports microbial production in the composting mixture. More intensive and faster humification is achieved if composting piles are inoculated with microorganisms by seeding new compost with some amount of mature compost. Compared to controls, composts inoculated with microorganisms stimulated degradation of hemicellulose, cellulose, and lignin by 28%, 21%, and 25%, respectively (Jurado et al., 2015). The greater the amounts of amino acids and reducing sugars produced during degradation, the higher the positive effect on HS synthesis (Wu et al., 2017).

Treatments A3, A4, and A6 had a dominant peak in the area representing lignin-like structures. These treatments contained large quantities of green waste (e.g., branches, leaves), shredded wood, straw, and paper, which are rich in cellulose and lignin and thus were included in the structure of HA. Bulking materials that are rich in cellulose and lignin can influence humification. These materials decompose more slowly and therefore can slow down composting processes and humification (Kulikowska & Sindrewicz, 2018).

Treatment A5 differed from other composts made from more traditional composting technologies. A5 contained frass from *H. illucens* larvae activity, as well as chitin and food residues accumulated during feeding, and the core structure of HA differed compared to other treatments. Although *H. illucens* larvae frass can be used as organic fertilizer, the fresh frass might contain phytotoxins, which decrease plant growth. To increase HA concentrations and frass stability, it is suggested to post-compost the frass (Lopes et al., 2022; Song et al., 2021).

The quality of composts is reflected by the content of both HS and nutrients. Although some composts may have lower nutrient concentrations, they may still be a valuable source of HS. Compost value depends in part on totP or labileP concentration. Compost A2 had the best value based on the combination of totP and percentage of TOC bound in HA and FA (Figure 8), but based on labile-P, the quality of A2 was poor compared to A1 and F3. Compost A3, with a high proportion of TOC bound in HA and FA, but a relatively low nutrient content (Figure 8, Table 6), could provide maximum fertilizing effects when used with mineral fertilizers. Combinations of low-nutrient-compost and mineral fertilizers can improve soil health and nutrient content to improve crop yields (Ibrahim et al., 2022; Issoufa et al., 2019). Such

composts can also benefit horticultural uses as a soil amendment or ingredient in growing media. Various composts can act similarly to peat moss, but with considerably lower costs and environmental impacts (Isaka et al., 2021; Raviv, 2005).

Although composts varied in HS concentrations, all HS will act as biostimulants and promote plant growth, regardless of origin (Klavins et al., 2021; Savy et al., 2020). Compared to chemical fertilizers, composts increase soil available nitrogen (N) and soil organic carbon (SOC) and improve soil fertility (Hu et al., 2018). These properties make composts a valuable organic fertilizer and soil improver that should be used more widely alongside chemical fertilizers.

Although chemical analyses are important in evaluating composts, it is also necessary to evaluate phytotoxicity and impacts on plant growth. For example, Garden cess (*Lepidium sativum*) is a sensitive indicator of compost toxicity (Kapanen & Itävaara, 2001). Declarations of plant response tests are necessary if the compost is to be used as a soil improver or mixing compound in growing media (Siebert & Auweele, 2014). Chemical analyses and plant test results together determine the effective dose of different composts.

# 7. CONCLUSIONS

A circular economy stresses the need for increased utilization and valuation of bio-waste, while it is reasonable to process waste and use the end-products locally. Composting is one possible solution for reusing bio-waste in Estonian conditions, but in temperate climates, seasonality must be considered. Composts based on different input materials can function as a soil improver by adding organic matter, beneficial bacteria, and humic substances to the soil, or even as organic fertilizer if nutrient concentrations in compost meet required levels. The selection of additives and adjustments to composting parameters can positively impact the properties of humic substances and nutrients in compost products.

The established hypotheses were confirmed:

- proximity composting of fish waste is technologically feasible. The biofilter effectively eliminated odour problems and required temperatures (> 70 °C for at least 1 hour) for handling animal by-products were achieved by all fish waste composting experiments. To ensure better mixing of input materials and microbiological activity, including additives, such as mature compost, peat, or lake sediment, is recommended (**I**).
- Composts made from different input materials have different relative proportions and concentrations of bioavailable P. Fish waste compost contained high concentrations of overall P, of which 57–66% was in potentially bioavailable form. For comparison, sewage sludge compost contained the highest concentration of overall P, of which only 6% was in potentially bioavailable form. In addition, fish compost contained a large amount of calcium bound P **(II**).
- All selected composts contained humic substances, including humic acids and fulvic acids, but their concentrations, properties, and relative proportions depended on input materials (**III**).

This PhD thesis highlighted the great potential of small bio-waste streams, which have not been considered to date. Such waste can be of high plant nutritional value, with the opportunity to produce high-quality composts that can be used to improve the physical and chemical

properties of soil, as well as to increase crop yields. Current work confirmed that composts can vary in quality and benefits for different purposes. Diversifying descriptions of compost quality parameters will educate the consumer about differences, benefits, and appropriate uses of compost for soil improvement and/or fertilizer usage.

Recommendations:

- Proximity composting in regional centres is recommended for more efficient processing of local bio-waste streams. Local communities or entrepreneurs should map such bio-waste streams in the area and encourage local composting facilities to produce high-quality product.
- The evaluation of composts as alternative P sources for agriculture should rely on relative distributions of P forms in the compost, in addition to total P concentration.
- At present, organic matter content is required by regulations but does not describe the composition of organic matter. To obtain additional information on the value of a particular compost, humic substances contents could be defined, in addition to organic matter contents. Emphasizing beneficial quality parameters of compost could provide a significant market advantage.

As the need for bio-waste management and utilization increases, future research should focus on mapping small scale waste streams and their composition. It is also necessary to raise awarness of both producers and consumers, while encouraging recycling of bio-wastes that have received little attention to date. Further research could also specify which forms of P, in addition to immediately plant-available labile P, can be converted to plant-available forms during longer time periods in different soil conditions. This knowledge could further advance one goal of the circular economy – converting bio-waste into a humus-like and nutrient-rich resource in a bioeconomy-based value chain.

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# SUMMARY IN ESTONIAN

## BIOJÄÄTMETE KOMPOSTIMINE RINGMAJANDUSE TINGIMUSTES

### Sissejuhatus

Kliimamuutused ja rahvastiku kiire kasv on suurendanud muret toiduga varustatuse ning toiduhindade stabiilsuse pärast (Schmidhuber & Tubiello, 2007). Sellest tulenevalt on Euroopa Liit (EL) võtnud vastu erinevaid meetmeid eesmärgiga kaitsta meie muldasid, pidurdada kliimamuutust ja tagada kõigile toiduga varustatus (European Commission, 2019).

ELi üks eesmärke on suurendada biojäätmete ringlussevõttu ning vähendada seeläbi 2030. aastaks toitainete kadu vähemalt 50% (European Commission, 2020a, 2021). Ainuüksi ELis toodetakse aastas 138 miljonit tonni biojäätmeid, mille ringlussevõtul saaks toota toitainerikkaid väetisi või mullaparandajaid (Severin et al., 2021). Eestis koguti 2021. aastal eraldi vaid 45 028 tonni biojäätmeid, millest ringlusse võetakse alla 30%. Samal ajal sattus segaolmejäätmetesse ligikaudu 100 431 tonni biojäätmeid (Taraskin, 2022).

Biojäätmete jätkusuutlikuks käitlemiseks on vaja neid liigiti koguda. Seetõttu nõutakse ELis, et biojäätmete kogumine muutuks alates 1. jaanuarist 2024 kõigile kohustuslikuks (Directive (EU) 2018/851). Lisaks soositakse biojäätmete käitlemist nende tekkekoha vahetus läheduses, mis aitaks vähendada transpordikulusid ning luua uusi võimalikke töökohti. Kompostimine on potentsiaalne viis biojäätmete käitlemiseks, muutes need toitainerikkaks tooteks, mida saab kasutada aianduses või põllumajanduses (Diaz et al., 2011; Lim et al., 2016). Kompost sobib kasutamiseks orgaanilise väetise või mullaparandajana, vähendades samal ajal mineraalväetiste kasutamise vajadust (European Commission, 2019).

Tavapärased kompostimisel kasutatavad materjalid on toidu- ja aiandusjäätmed, sõnnik ning reoveesetted (Lim et al., 2016). Kompostimiseks sobivad ka kalajäätmed, kuid kiire riknemise ja ebameeldiva lõhna tõttu kasutatakse neid vähe (Harrington et al., 2005; Radziemska et al., 2019). Kalajäätmete alla liigitub ka majanduslikult

tähtsusetu kaaspüük, mida kalurid praegu tasuva alternatiivi puudumisel veekogudesse tagasi viskavad (Harrington et al., 2005). Võrtsjärve näitel on arvutatud, et 1 kg kaubakalaga esineb koos 1,3 kg kaaspüüki; samas on need kogused liiga väikesed, et toota biogaasi või kalajahu (Silm et al., 2020). Kui kaaspüük piirkondlikult kokku koguda, võivad kogused olla kompostimiseks piisavalt suured.

Kompostimine on traditsiooniline biojäätmete käitlemise viis, mida võib teha nii tööstuslikult reaktorites või väljas aunades kui ka kodumajapidamistes kompostikastides. Tööstuslikult kompostides tuleb järgida ohutusnõudeid, mis sätestavad, et protsessi käigus peab temperatuur püsima kolme päeva jooksul üle 65 °C või kümne päeva jooksul üle 55 °C (Regulation (EU) 2019/1009; Siebert & Auweele, 2014), loomsete kõrvalsaaduste korral 1h jooksul üle 70 °C (Commission Regulation (EU) 142/2011). Sel viisil tagatakse, et valmis kompost ei sisaldaks umbrohuseemneid ega ohtlikke patogeene. Kompost sisaldab taimedele kättesaadavaid toit- ja orgaanilisi aineid, mis aitavad parandada mulla süsinikusisaldust, struktuuri, veehoidmise võimet ning taastada huumusesisaldust. Seeläbi aitab komposti kasutamine kaasa mulla viljakuse säilimisele (Barral et al., 2011; Khater, 2015; Milinković et al., 2019).

Olenevalt materjalidest võivad kompostid olla väga erineva kvaliteedi ja toitainesisaldusega. Kompostide iseloomustamisel on saanud vähe tähelepanu fakt, et need sisaldavad peale toitainete ka mulla kvaliteeti parandavaid humiinaineid (Guo et al., 2019; Mosa et al., 2020). See on tähtis, sest on näidatud, et 45% ELi muldadest on liiga väikese orgaanilise aine sisaldusega ning toimub üleüldine mulla kvaliteedi halvenemine (European Commission & European Environment Agency, 2012).

## Püstitatud hüpoteesid ja eesmärgid

Doktoritöö raames töötati välja suunised seni kasutamata kalajäätmete väärindamiseks kohalikul tasandil, et muuta need kompostimise teel orgaaniliseks väetiseks või mullaparandajaks. Selleks sõnastati järgmised eesmärgid:

- leida sobilik lahendus kalajäätmete ja kaaspüügi kompostimiseks nende tekkekoha läheduses ning muuta need toitainerikkaks väetiseks või mullaparandajaks (**I**);
- hinnata kalajäätmete komposti fosforisisaldust ja -vormide jaotumist; võrrelda saadud tulemusi teistest jäätmetest valmistatud kompostidega (**II**);
- hinnata humiinainete sisalduse ja omaduste sõltuvust kompostimaterjalidest (hobusesõnnik, reoveesetted, kalajäätmed, toidujäätmed, haljastusjäätmed) (**III**).

Olemasolevate teadmiste ja varasemate uuringute põhjal püstitati järgmised hüpoteesid:

- kalajäätmete kompostimine jäätmete tekkekohas on tehnoloogiliselt võimalik ja võib kohalikul tasandil toetada üleminekut ringmajandusele **(I);**
- kalajäätmed sisaldavad suures koguses fosforit (P), millest enamik on taimedele omastatavas vormis (**II**);
- humiinainete sisaldus ja omadused sõltuvad otseselt kompostitavast materjalist (**III**).

## Katsematerjal ja metoodika

### Kompostide tähistamine

Doktoritöö aluseks olevates artiklites on samu komposte märgitud erinevate tähistega. Selguse huvides on antud töö raames kompostidel uued tähised (tabel 1).

**Tabel 1.** Kompostide tähised.

| Uus tähis | Jäätmed | Töötlus | Artikkel I | Artikkel II | Artikkel III |
|---|---|---|---|---|---|
| F1 | Kalajäätmed, kaaspüük | Aunkompostimine | F1 | FW1 | - |
| F2 | Kalajäätmed, kaaspüük | Aunkompostimine | F2 | FW2 | - |
| F3 | Kalajäätmed, kaaspüük | Aunkompostimine | F3 | FW3 | C1 |
| F4 | Kalajäätmed, kaaspüük | Aunkompostimine | F4 | - | - |
| F5 | Kalajäätmed, kaaspüük | Aunkompostimine | F5 | - | - |
| A1 | Hobusesõnnik | Aunkompostimine | - | HM | C2 |
| A2 | Reoveesete | Aunkompostimine | - | SS | C3 |
| A3 | Munitsipaalalade haljastusjäätmed | Aunkompostimine | - | GW | C4 |
| A4 | Toidujäätmed (kanajäätmed) | Aunkompostimine | - | - | C5 |
| A5 | Kohviku ja kaubanduse jäätmed | *H. illucens* töötlus | - | - | C6 |
| A6 | Toidujäätmed | Vermikompostimine | - | - | C7 |

A5 on kompostimata *H. illucens* sõnnik.

### Kalajäätmete aunkompostimise katse (I)

2018.–2019. aasta kevad- kuni sügisperioodil valmistati viis 10 $m^3$ suurust kalakomposti auna, mis erinesid algmaterjalide koguste, lisaainete ning kompostimisperioodi poolest (tabel 2). Kõik kompostid sisaldasid kalajäätmeid (peamiselt Võrtsjärvest püütud kaaspüük) ja nisupõhku. Kompostisegud valmistati nii, et süsiniku ja lämmastiku suhe (C/N) oleks 30:1. Selleks mõõdeti nii põhu kui ka kala süsiniku- ja lämmastikusisaldus. Lisaks segati erinevatele kompostisegudele juurde valmis komposti, turvast ja savirikast järvesetet, et hinnata nende mõju kompostimisprotsessi tõhususele.

**Tabel 2.** Kalakompostimise katse. Aktiivne periood (p) näitab päevades, kui kaua oli kompost aunades.

| Tähis | Jäätmed | Lisaaine | Skaala | Vastavus kvaliteedi regulat-sioonidele | Aktiivne kom-postimise periood (p) |
|---|---|---|---|---|---|
| F1 | Kaaspüük, kalatööstuse jäätmed | Põhk | Tööstuslik | Jah | 185 |
| F2 | | Põhk | Tööstuslik | Jah | 70 |
| F3 | | Põhk, turvas, valmis kom-post | Tööstuslik | Jah | 50 |
| F4 | | Purustatud põhk, järvesete | Tööstuslik | Jah | 135 |
| F5 | | Purustatud põhk, järvesete, valmis kom-post | Tööstuslik | Jah | 135 |

Kompostiprotsessi seirel jälgiti aunades automaatsete logeritega temperatuuri ja määrati käsitsi kaks korda päevas süsihappegaasi ($CO_2$) sisaldust.

Kuna kalajäätmete lagunemisel tekkis ebameeldiv lehk, ehitati teise aunkompostimise (F2) katse alguses biofilter, mida kasutati edukalt ka järgmistes kalajäätmete kompostimise katsetes (F3–F5).

Kõigist kompostidest koguti proovid, mida kuivatati temperatuuril 105 °C ja jahvatati. Kogutud proovidest tehti keemilised analüüsid fosfori- (P), kaaliumi- (K), kaltsiumi- (Ca), magneesiumi- (Mg) ning süsiniku- (C) ja lämmastikusisalduse (N) määramiseks.

Edasistes katsetes võrreldi kalajäätmete komposti kuue alternatiivse kompostiga. Neli neist olid valmistatud aunkompostimise tehnoloogiaga ja koosnesid vastavalt hobusesõnnikust, reoveesetetest, haljastusjäätmetest või toiduainetööstuse jäätmetest (kanajäätmed). Lisaks valiti toidujäätmete käitlemisel saadud ogakärblase (*H. illucens)* vastsete kompostimata sõnnik ja toidujäätmetest valmistatud vermikompost.

## Fosforivormide määramine (II)

Fosforisisalduse ja -vormide määramisel kasutati järjestikuse ekstraheerimise meetodit (Rydin, 2000). Vastav analüüs tehti 2018. aasta kalakompostide (F1-F3) ja hobusesõnniku, reoveesetete ning haljastusjäätmete kompostidest (A1-A3). Antud meetodil määrati kuus P vormi:

Labiilne P – veeslahustunud või nõrgalt seotud P, koheselt taimedele omastatav;
Fe-P – redutseerivate metallidega seotud P (Fe, Mn);
Al-P – alumiiniumiga seotud P (Al);
Org-P – orgaanilise ainega seotud P;
Ca-P – kaltsiumiga seotud P (Ca);
SumP – ekstraheeritud fraktsioonide summa. mis erineb üldfosforist nn jääkfosfori võrra.

## Humiinainete määramine (III)

Humiinainete analüüsideks valiti võimalikult erineva koostisega kompost: kalajäätmete (F3), hobusesõnniku (A1), reoveesetete (A2), haljastusjäätmete (A3) ja kanajäätmete (A4) kompostid, samuti ogakärblase vastsete sõnnik (A5) ning biosütt sisaldav vermikompost (A6). Humiinhapete lahuses määrati neeldumisspekter lainepikkusel 230–670 nm. Veel määrati humiinhapete (HA) ja fulvohapete (FA) orgaanilise süsiniku üldsisaldus (TOC), mille põhjal arvutati nende suhe (HA/FA). HA infrapunaspektrid määrati Fourier' teisenduse infrapunaspektromeetri (FTIR) abil, kasutades lainearve 400–4000 $cm^{-1}$. Fluorestsentsi ergastus-emissioonispektrite (EEM) abil määrati HA ja FA omadusi, kasutades ergastuslainepikkust 250–600 nm ja emissioonilainepikkust 250–600 nm.

## Tulemused ja arutelu

Kompostimise katsest selgus, et kalajäätmeid saab edukalt käidelda aunkompostimise teel. Kõikides valmistatud aunades ületas temperatuur vähemalt 1h jooksul 70 °C, mis on määruse kohaselt loomsete kõrvalsaaduste käitlemiseks vajalik temperatuur (Commission Regulation (EU) 142/2011). Kuna esimeses kompostikatses tekkis kalade lagunemisel ebameeldiv lehk, ehitati biofilter, mis lahendas edukalt selle probleemi.

Biofiltri käivitamiseks kasutati ventilaatorit, mis suunas kompostiaunast pärineva õhu sundventilatsiooni abil läbi õhufiltri. Biofiltri tööks vajalik ventilatsioon aitas ka komposti hapnikuga varustada, vähendades esimestel päevadel auna segamise vajadust. Katse käigus selgus, et sellise väikesemahulise kompostiauna puhul oli optimaalne biofiltri rakendusaeg kaks korda päevas 15 minutit. Lõhnaprobkeem kadus esimese 4–5 päeva jooksul. Erinevatest katsetest selgus, et põhu purustamine ja selliste ainete nagu valmis komposti, turba või savirikka järvemuda lisamine aitab luua kompostimiseks vajalikele mikroobidele paremaid keskkonnatingimusi. Seetõttu võib järvede tervendamise käigus eemaldatud savirikkal mudal olla potentsiaali kasutamisel kompostis lisaainena.

Fosforivorme määrati kalajäätmete (F1–F3), hobusesõnniku (A1), reoveesetete (A2) ja haljastusjäätmete (A3) kompostidest. Selgus, et kõik analüüsitud kompostid sisaldasid erinevas kontsentratsioonis summaarset fosforit (SumP), kuid see ei kirjelda fosfori kättesaadavust taimedele. Fosfori fraktsioneerimise käigus selgus, et kõik kompostid erinesid ka fosforivormide poolest. Kalakompostid (F1–F3) ja hobusesõnniku kompost (A1) sisaldasid enim labiilset fosforit, mida taimed omastavad. Kalakompostid (F1–F3) sisaldasid summaarset fosforit rohkem kui hobusesõnniku kompost (A1), kuid 1/3 sellest oli seotud kaltsiumiga, mis pärines ilmselt kalaluudest. Kõige rohkem summaarset fosforit sisaldas reoveesetete kompost (A2), kuid sellest vaid 6% oli labiilses, taimedele kohe omastatavas vormis. Haljastuskompost (A3) sisaldas kõige vähem summaarset fosforit ja suur osa sellest (57%) oli seotud kaltsiumiga, mis pärines ilmselt haljastusjäätmete kogumisel nende hulka sattunud paekiviosakestest.

Humiinainete analüüsid näitasid, et kõik kompostid sisaldasid erinevas koguses humiin- ja fulvohappeid, mille kontsentratsioon sõltus kompostimaterjalidest. Komposti küpsust näitav humiin- ja fulvohapete massisuhe HA/FA oli suurim hobusesõnniku (A1; 7.88) ning haljastusjäätmete (A3; 7,82) kompostides, väikseim aga ogakärblase vastsete sõnnikus (A5; 1,46) ja kanajäätmete (A4; 1,42) kompostis, mis sisaldasid samas kõige rohkem rasvu (vastavalt 2,1% ja 4,2%). EEMi analüüs näitas, et kompostid sarnanesid fulvohapete spektri poolest, milles oli tuvastatav polüsahhariidide piik. Suuremad erinevused olid humiinhapete jäljes. Erinevad tugiained, nagu põhk, oksad ja lehed, mõjutasid humiinhapete jälge. Ogakärblase vastsete sõnnik (A5) erines teistest kompostidest humiinhapete spektri poolest.

## Kokkuvõte

Antud töö tõi välja väikeste biojäätmevoogude tähtsuse. Sellised Eestis vähe tähelepanu saanud biojäätmed võivad olla taimedele vajaliku suure toitainesisaldusega ja sobivad seetõttu kvaliteetse toote valmistamiseks. Üks võimalus neid edukalt käidelda on kompostimine, mis sobib lihtsuse ja paindlikkuse tõttu jäätmekäitluseks tekkekoha läheduses. Töö kinnitas, et erinevatest biojäätmetest valmistatud kompostid võivad olla väga erineva kvaliteediga. Seetõttu julgustatakse kompostitootjaid oma komposte põhjalikumalt hindama ning harima tarbijaid kompostide erinevuse ja kasutusvõimaluste kohta.

Doktoritöös leidsid kinnitust järgmised hüpoteesid:

- Väikesemahuliste kalajäätmevoogude määrusekohane kompostimine tekkekoha läheduses on võimalik. Kõikides kompostiaunades saavutati määrusega nõutud temperatuur (> 70 °C vähemalt 1h jooksul), mis on vajalik kahjulike patogeenide ja umbrohuseemnete hävitamiseks. Biofilter aitas tõhusalt kõrvaldada kala lagunemisel tekkiva ebameeldiva leha ja õhutas samal ajal aunu. Kompostimisprotsess oli edukam, kui kalale ja põhule lisati auna moodustamisel juba valmis komposti, turvast või järvesetteid **(I).**
- Kõik töös uuritud kompostid erinesid fosforisisalduse ja -vormide jaotumise poolest. Kalajäätmetest valmistatud kompostis on suur fosforikontsentratsioon, millest 57–66% oli taimedele omastatavas vormis. Võrdlusena võib tuua reoveesetete komposti, mis sisaldas kõige rohkem fosforit, kuid sellest taimedele kättesaadav oli vaid 6% **(II)**.
- Kompostimisel kasutatavad materjalid mõjutavad otseselt humiinainete sisaldust ja omadusi. Kõik uuritud kompostid sisaldasid humiinaineid, sh humiin- ja fulvohappeid, kuid viimaste omavaheline suhe oli kompostides erinev **(III)**.

Soovitused:

- Biojäätmete tõhusamaks käitlemiseks on soovitatav luua kohalikul tasandil võimekus biojäätmete kompostimiseks, et muuta need väärtuslikuks põllumajanduslikuks ressursiks.

- Komposti kvaliteedi hindamisel on lisaks üldisele fosforisisaldusele soovitatav määrata ka taimedele omastatava fosfori osakaalu vorme.
- Komposti kui toote turustamisel on kasulik määrata peale orgaanilise aine sisalduse ka humiinainete sisaldus ja rõhutada selle kasutegureid.

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# I

# ORIGINAL PUBLICATIONS

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# Open windrow composting of fish waste in Estonia

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**Abstract.** By-catch fish is caught unintentionally during the fishing and is currently thrown back in water bodies to cause the water pollution. Currently fishermen does not have a motivation to bring the by-catch fish to the shore, as it needs to be sorted by fish species, causing fishermen extra work without additional income. Estonian Ministry of Rural Affairs decided to give funding to present study with purpose to find solution to this matter. One possible solution for by-catch fish utilization is to produce high value nutrient rich fertilizer in order to close nutrient cycle and return valuable nutrients into soil. The adaptive study of outdoor windrow composting was conducted with consecutive treatments, rather than simultaneously, in order to make adaptive improvements to the set-up of each consecutive treatment. The consecutive treatments showed that fish waste composting is manageable from a technical perspective, feasible in a temperate climate, and that this type of compost holds high potential as an organic fertiliser or soil improver. Composting process started rapidly and, as required by the EU Commission regulation EU 142/2011, temperatures exceeded 70 °C for at least 1 h in all windrows. While initial treatments suffered from odours, as well as events inhibitive to the composting process, these disadvantages were successfully avoided in later treatments by adding a biofilter and inoculant from previous composting windrows, as well as lake sediments. Rather than disposing of low-value fish, these can be recycled into stable and nutrient-rich compost on-site, near fishing harbours.

**Key words:** by-catch, biofilter, clay minerals, lake sediment, organic fertiliser, wheat straw.

## INTRODUCTION

Composting is an aerobic microbial process that turns organic material into stable humus-like material that can be used to improve soil texture and nutrient content for agricultural use. Composting is suitable for treating different kinds of organic wastes such as food waste, manure, green wastes or municipal solid wastes (Rynk, 1992;

Awasthi et al., 2020). Composting can be performed with different technologies, and at different scales (Rynk, 1992; Hobson et al., 2005). In Estonia, composting is mostly done in open windrows, which are periodically mixed in order to improve porosity and oxygen supply. Mainly source-separated municipal biowaste is composted in Estonia (BEU, 2020), sometimes in combination with garden waste and animal by-products. National end-of-waste regulations for biowaste compost (BEU, 2020) and sewage sludge compost quality (Regulation, 2017) have been enforced since 2013 and 2017, respectively.

Nutrient rich compost holds high potential as an organic fertiliser or soil improver, offering the possibility to reduce the use of synthetic fertilisers. Although fish waste composting is not yet included in local waste management plans in Estonia, there is growing interest for fish waste valorisation and associated nutrient recycling. The topic was also prioritised by the Estonian Ministry of Rural Affairs, through the decision to fund the present study, with the aim of finding new solutions regarding fish waste management. Both the waste from fish processing, as well as by-catch, could represent inputs for composting. For example, Lake Võrtsjärv represents approximately 8% of commercial fishing in Estonian lakes and rivers. In 2018, it was estimated that, for each kg of commercial catch, there were approximately 1.45 kg of by-catch, resulting in 6,300 tons of by-catch per year (Bernotas et al., 2018). During the years 2008–2017, the yearly average commercial catch for Estonian lakes and rivers was 2,800 tons, 65,000 tons for the Baltic Sea and 12,500 tons for the Atlantic Ocean (Quarterly bulletin…., 2018). In Estonia, similar to Baltic region as a whole, fisheries represent an important part of the economy; thus, fish waste composting should be included in local waste management plans.

Besides leftovers from the fish industry, a significant source of unwanted fish is by-catch. By-catch includes fish caught of unusable size, damaged fish and species without economic value (Clucas, 1997). Rather than being revalued, by-catch in Estonia is often thrown back into the aquatic environment, where it results in pollution.

Long-term cultivation and fertilisation both affect the humus content in soils (Hospodarenko et al., 2018). Also, mineral sources for phosphorus (P) fertilisers are located in sensitive regions, or generate a large ecological footprint during transportation to agricultural fields (Roy et al., 2005). Therefore, the available of local P nutrient sources is vital. Compost can add humus to soils over a longer period, and reduce the need for using synthetic fertilisers (Epstein, 2017). Compost represents a slow-release fertiliser, as nutrients from composted material are released slowly yet consistently, thus building up a stable reserve of soil nutrients (Hepperly et al., 2009).

It has been claimed that fish-derived amendments behave like slow-release nitrogen (N) fertilisers, and hold higher P release capacities, as well as being rich in calcium (Ca) (Laos et al., 2000). Therefore, fish waste could be processed into a nutrient-rich soil amendment. There have been different composting trials indicating that fish waste compost represents a suitable fertiliser for agricultural use, and can increase crop yield (Laos et al.,1998; López-Mosquera et al., 2011; Illera-Vives et al.,2015; Radziemska et al., 2018).

Due to problematic odour emission, fish waste must be stabilised rapidly. One key factor may be choosing an appropriate compost mixture. In addition to organic wastes, different substrates such as bulking agents and other additives must be used while creating compost windrows. Shredded wood and straw are porous and rich in carbon

(C), and can improve aeration and moisture regulation, as well as improve odour absorption (Rynk, 1992). Sufficient aeration is necessary, as a limited oxygen ($O_2$) supply during the active composting period decreases microbial activity, inhibiting the composting process (Avnimelech et al., 2004). To understand the progression of composting, $O_2$ consumption must be measured. Respiration rate reflects microbial activity and the decomposition of organic matter. Accordingly, higher $O_2$ consumption is associated with higher microbial activity. In stable or mature compost, easily degradable organic matter is decomposed, and the resulting compost is safe to use (Wichuk & McCartney, 2013). Moisture content is also important, as water transports dissolved nutrients to microorganisms, resulting in greater microbial activity, which is essential for the decomposition process.

Straw as a bulking agent is light and fluffy, making the windrows porous. Also, it biodegrades more easily than wood chips. This may be the result of higher undesirable ammonia ($NH_3$) emission; but at the same time, it has been demonstrated that composts with straw amendments contain higher nutrient concentrations than those with wood chips (Sardá et al., 2019).

In order to improve the composting process, different additives such as biochar, peat, clay minerals and mature compost can be added into fresh composting mixtures, with the purpose of reducing gas- and odour emissions, as well as to increase the nutrient content of the final product, thus influencing microbial activity. As a result, the degradation process starts earlier and the thermophilic phase lasts longer (McCrory & Hobbs, 2001; Gabhane et al., 2012; Sánchez-García et al., 2015; Barthod et al., 2018). Additives such as clay minerals can also limit water loss and reduce greenhouse gas emissions (Barthod et al., 2016). It has been shown that an adequate moisture content has have a greater influence than temperature on the decomposition process (Liang et al., 2003); an adequate moisture level in compost is approximately 40–60% (Rynk, 1992). Clay minerals can also influence microbial activity, as clay provides a pH suitable for sustainable microbial growth (Stotzky & Rem, 1966).

Temperature is an important indicator for the composting process, reflecting both the degradation of organic matter and microbial activity (Eklind et al., 2007). The thermophilic phase occurs when temperatures inside compost windrows exceed 40 °C; where the organic matter decomposition process is the fastest at 55 °C (Eklind et al., 2007; Rynk, 1992), when microbial activity is highest (Miyatake & Iwabuchi, 2005).

Compost must be safe for humans and the environment, and therefore it must be free of pathogens. Because of its status of being animal by-products (ABPR), temperatures must exceed 70 °C for 1 h for sanitation purposes, as required by the EU Commission regulation EU 142/2011 (European Commission, 2011). After the active composting period, it is necessary to let compost mature, as immature compost may inhibit plant growth (Ozores-Hampton et al., 2002).

The aim of this study was to develop a practical technological solution for composting, whereby fishermen can use nearby fishing harbours to revalue by-catch fish, allowing this resource to be converted into nutrient rich fertiliser suitable for agricultural use. We focused particularly on solutions for reducing odours and meeting sanitation requirements.

## MATERIALS AND METHODS

### Composting experiment

The composting experiment was conducted in Taali, Pärnu County, Estonia. Five batches of fish compost (corresponding to treatments F1–5), each having different raw material ratios, were composted from spring 2018 to autumn 2019 (Table 1). Treatments were performed consecutively, rather than simultaneously, in order to make adaptive improvements to the set-up of each consecutive treatment. The composting site was on a concrete surface, in order to prevent soil- and groundwater pollution. All windrows were covered with the semi-permeable geotextile KSV 200 (Compost Systems, Austria), in order to reduce the weather impact, maintain the in-compost microclimate, and protect the windrows from animals. All treatments were carried out at a scale 10 $m^3$ (12–16 m long and 1.5 m wide windrows). The main component in each compost mixture was low-value by-catch fish from Lake Võrtsjärv, followed by wheat straw as bulking material, as it is considered easily available.

**Table 1.** Initial ingredients of compost mixtures (kg). F1–5 represent different treatments (windrows). An asterisk (*) indicates that the straw was shredded

| Windrow | By-catch fish | Straw | Water | Peat | Mature compost (horse manure) | Mature compost (fish, F1 treatment) | Lake sediment |
|---|---|---|---|---|---|---|---|
| F1 | 4,406 | 1,125 | 0 | 0 | 0 | 0 | 0 |
| F2 | 1,705 | 1,312 | 1,860 | 0 | 0 | 0 | 0 |
| F3 | 930 | 943 | 1,996 | 960 | 913 | 545 | 0 |
| F4 | 905 | 1,155* | 1,150 | 0 | 0 | 0 | 355 |
| F5 | 960 | 1,347* | 1,200 | 0 | 0 | 460 | 180 |

For treatments F1, F2 and F3, unshredded wheat straw was used; whereas in treatments F4 and F5, wheat straw was shredded with the trailed straw chopper PRIMOR 5570 M (KUHN Group, Austria).

Additives such as peat, mature compost (used as inoculant), and lake sediment rich in clay minerals, containing 6 g of clay material per 100 g dry matter (DM), were used in different treatments. All by-catch and lake sediment originated from Lake Võrtsjärv. Straw was collected in 2017 from the surrounding agricultural fields in in Taali, Pärnu County, Estonia. The straw was subsequently dried and pressed into 250 kg rolls. The by-catch fish samples, and straw, were analysed for total C and N in order justify an appropriate balance of material inputs. The calculations were based on the assumption, that efficient composting process takes place, when C/N is (20–30)/1. Composting materials were mixed together using a BVL van Lengerich feed mixer V-Mix 13 2S plus (BVL Group, Germany).

Different treatments lasted for different lengths of time, as treatments F2 and F3 were conducted during autumn, resulting in shorter period of time suitable for maturation process (Table 2).

**Table 2.** Composting treatments. F1–5 represent different treatments (windrows)

| Windrow | Composting duration (d) | Moisture adjustment | Moisture adjustment | Turning frequency |
|---|---|---|---|---|
| F1 | 185 | No | No | Day 35 |
| F2 | 70 | Yes | Days 1, 9 | Days 9, 11, 14, 16 and 19 |
| F3 | 50 | Yes | Day 1 | Days 5, 8, 11 and 22 |
| F4 | 135 | Yes | Days 1, 9 | Days 6, 9, 15, 18, 25 and 40 |
| F5 | 135 | Yes | Days 1, 9 | Days 6, 9, 15, 18, 25 and 40 |

Temperature was measured automatically from windrows F2, F3, F4 and F5, using thermocouple wires (type K, min -10 °C, max 105 °C, 2-Core, Unscreened, PVC-insulated, 100 m; Thermosense, UK). Depending on treatment, 2 or 3 sensors were inserted into each windrow, approximately 20 cm up from concrete surface. Carbon dioxide ($CO_2$) concentration was measured manually twice a day, using a Brigon Testoryt $CO_2$ detector (0–20%; Brigon Messtechnik, Germany). When temperatures exceeded 70 °C for several days, or $CO_2$ concentration rose above 10%, windrows were mixed using either a Backhus 16.30 windrow turner (Eggersmann, Germany) or an ST 300 windrow turner (COMPOST and WASTE technology, Austria).

To prevent odours, a biofilter was associated with each treatment (Fig. 1). The biofilter was made from a 1 $m^3$ container filled with a 4:1:1 ratio of shredded wood, coal and mature compost of horse manure, respectively; and was connected to its respective windrow via a 75 mm polyethylene aeration pipe perforated with 5 mm diameter holes spaced 10 cm apart, the holes forming two shifting lines. Negative pressure via fan was used for ventilation. Biofilters were turned on twice a day for 15 min, until problematic odours discontinued (4 to 5 days). The same biofilter was used for each treatment.

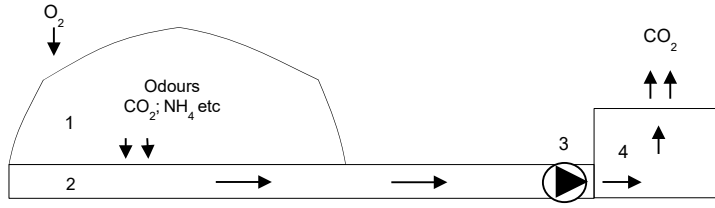


**Figure 1.** Biofilter- and windrow schematic: 1 – compost windrow; 2 – perforated pipe under the windrow; 3 – fan; 4 – biofilter.

**Chemical analyses**

Compost samples were collected from all treatments, from three different locations approximately 20–30 cm into the windrow. From each sample, approximately 250 g was homogenised, dried at 105 °C and ball-milled prior to analysis. DM- and ash concentrations were measured. P, potassium (K), Ca and magnesium (Mg) content was determined using the ammonium lactate (AL) method from Egnér et al. (1960); and C and N content was analysed using a VarioMAX CNS analyser (ELEMENTAR, Germany).

**Compost stability**

Four-day cumulative oxygen consumption was determined via manometric respirometry (OxiTop, WTW, Germany) according to the manufacture´s recommendations. Measurements were collected in 1 L air-tight containers with 50–60 g of fresh sample. Since produced $CO_2$ was removed by absorber (2 M NaOH), the oxygen consumption of samle could be calculated from the pressure drop in the container. Measurements were collected in triplicate at 20 °C in a climate chamber (Sanyo MLR-351H, Osaka, Japan), in total darkness.

**Statistical analyses**

A three-way analysis of variance (ANOVA) was performed in order to examine the effect of treatment, ambient temperature, duration of composting, and the interactions between these factors, on composting temperature. A one-way ANOVA was used to study the effect of treatment on mean temperature. A post-hoc Tukey *HSD* analysis was performed for pairwise comparisons, using the package 'agricolae' (Mendiburu, 2015) in R.

## RESULTS AND DISCUSSION

**Composting process**

In all treatments, the thermophilic phase was reached in 1 to 2 days, and temperatures exceeded 70 °C, as required by the EU Commission regulation EU 142/2011 on animal by-products. The duration of thermophilic phase was treatment-dependent. After the thermophilic phase, temperatures decreased rather quickly, and ambient levels were reached within days (Table 3).

**Table 3.** Temperature characteristics: n.d. – data not determined; * – temperature fluctuation. In F2 treatment temperature decreased < 35 °C several times since day 6 and rised again after mechanical mixing with windrow turner

| Batch Windrow | Time to reach > 45 °C | Number of days temp > 45 °C | Number of days temp > 70 °C | Peak temp °C | Time to reach the peak temp | Time to drop to ambient level < 35 °C |
|---|---|---|---|---|---|---|
| F1 | n.d | n.d | n.d | n.d | n.d | n.d |
| F2 | Day 1 | 14 | 1 | 72.9 | Day 1 | Day 6* |
| F3 | Day 2 | 21 | 7 | 76.4 | Day 6 | Day 23 |
| F4 | Day 1 | 63 | 18 | 75.0 | Day 7 | Day 63 |
| F5 | Day 2 | 69 | 21 | 78.6 | Day 8 | Day 73 |

In treatment F1, a windrow turner was used for mixing the compost ingredients. We do not recommend this method for preparing compost mix with fish, as whole fish were thrown from the windrow.

In deeper layers of the F1 windrow, fish started to slow-cook as a result of high temperatures; and in outer layers of this windrow, fish became either dried or rotted. To prevent the spread of odours, the F1 windrow was covered with peat for 2 weeks, starting on day 7. It was concluded that the wheat straw is suitable as bulking material, but as it is too dry, water should be added at the beginning of the composting process. Also, shredding of fish would speed up degradation. As the feed mixer later partly shredded the fish, it made the compost mixture more homogeneous.

When compiling the F2 treatment, water was added to the compost mixture. Since, due to fish decomposition, there was a considerable problem with fish odour emission from treatment F1, a biofilter was implemented in treatment F2. Initially, the biofilter was turned on for 15 min every 2 h, which caused the temperature drop in the windrow. It was concluded that the optimal biofilter working mode was twice a day for 15 min. The biofilter also provided a sufficient $O_2$ supply to windrow, thus reducing the need for mechanical mixing of windrows during the first days.

In treatment F2, the thermophilic phase was reached at the end of the day 1. For unknown reasons, the temperature started to decrease after day 5 (Fig. 2). After the temperature decreased, the windrow was mixed with the windrow turner. This resulted in a temporary rise in temperature. Temperatures in the treatment F2 windrow exceeded the thermophilic phase threshold for 16 days (Table 3). These high temperatures in compost are associated with microbial activity, and therefore temperature decreases are frequently influenced by low microbial activity (Alves et al., 2019; Wichuk & McCartney, 2013). This may have been the cause of temperature fluctuation in the treatment F2 windrow. However, the cause of low microbial activity is unclear, as easily-biodegradable substrate was not consumed until the end of the active composting period, and neither $O_2$ supply nor moisture content were not considered to be limiting factors. Further research is needed to conclude what causes these types of temperature fluctuations.

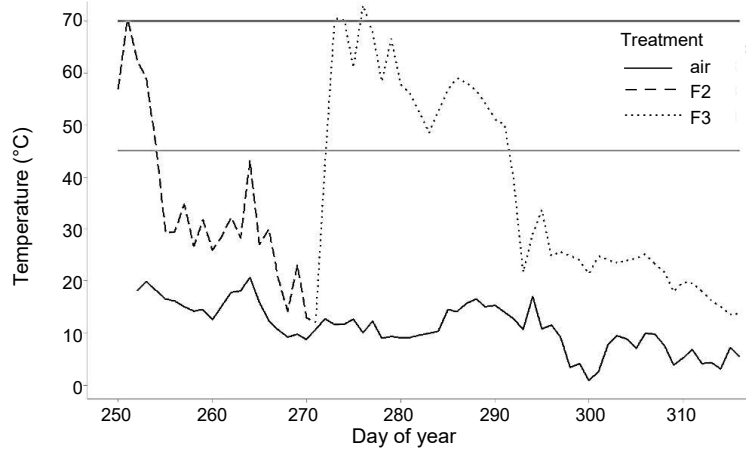


**Figure 2.** Windrow F2 and F3 temperature graphs from year 2018.

It was previously shown that the addition of mature compost can slightly increase temperature in the thermophilic phase (Maeda et al., 2009). For the purpose of stabilising temperatures during the active composting period, as well as to add more beneficial bacteria necessary for the decomposition process, peat and mature compost were added to treatment F3. The thermophilic phase for this treatment was reached by day 3, and temperatures were stable during the active composting period, which lasted for 23 days. As temperatures inside compost are not uniform (Avnimelech et al., 2004), the treatment

F3 windrow was mixed during days 5 and 8, in order to homogenise temperature and moisture. After turning the compost, temperatures inside the F3 windrow decreased for an hour, but then started to increase again. Similar behaviour has registered by Marešová & Kollárová, (2010). In their study they also noticed, that after mechanical mixing temperature decreased in the windrow, but after some time temperature started to increase, exceeding the previous level.

For treatments F4 and F5, both windrows contained by-catch fish, shredded straw and lake sediment; but in treatment F5, mature compost was also added. The mean in-windrow temperatures, between treatments F4 and F5, were not statistically different, even though the temperatures in F5 were slightly higher than in F4 (Fig. 3). In windrows of both F4 and F5, the thermophilic phase was reached by the end of day 1. Furthermore, temperatures > 65 °C lasted longer than in the previous treatments. In treatment F2 and F3, the thermophilic period lasted 14 and 21 days, respectively; while in treatments F4 and F5, the thermophilic period lasted 63 and 69 days, respectively (Table 3). Temperatures decreased to ambient levels in treatment F4 after day 63, and in F5 after day 73. While it has been reported that the decomposition process is most efficient at 55 °C (Eklind et al., 2007), our temperatures exceeded 70 °C for a long period, yet decomposition was still efficient. However, it should be considered that gas emissions can be more intense at higher temperatures (Eklind et al., 2007), and the metabolic activity of many microorganisms will decrease when temperatures exceed 60 °C (Miyatake & Iwabuchi, 2005).

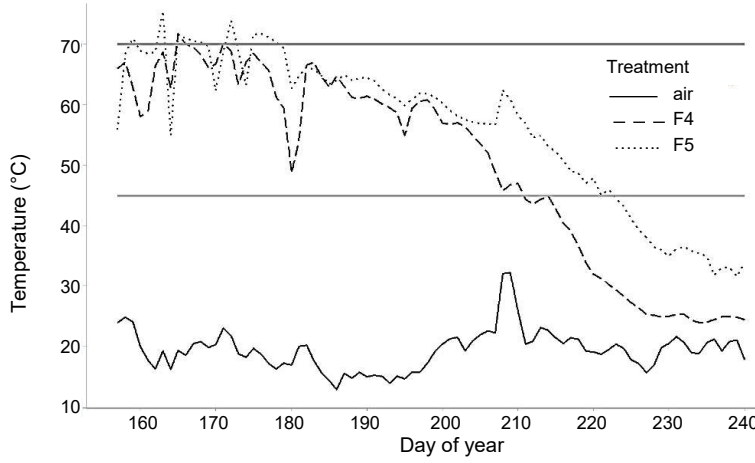


**Figure 3.** Windrow F4 and F5 temperature graphs from year 2019.

A more efficient composting process in treatments F4 and F5 can be attributed to the addition of shredded straw, which is readily degradable. In the first treatments (F1, F2 and F3), unshredded straw did not completely degrade, and was visible in the final compost product. Straw contains a large fraction of cellulose (29–35%), hemicellulose (26–32%) and lignin (16–21%) (Kapoor et al., 2016), for which decomposition is relatively slow (Eklind et al., 2007). Another explanation for more active composting

process can be explained with the addition of clay minerals from lake sediments. Compost constituents were better bound together, making the mixture more homogeneous and providing better conditions for decomposition. It has been shown that the addition of clay minerals can increase water retention capacity and binding the organic material of composting mass (Chen et al., 2018). Therefor adding clay minerals assures the better conditions for microbial activity, resulting in higher temperatures and a longer duration of the thermophilic phase.

**Compost stability**

According to the Estonian regulation for sewage sludge compost, compost is referred as stable if $O_2$ consumption over 4 days is less than 10 mg $O_2$ $g^{-1}$ DM (Regulation, 2017). These values in treatments F2 and F3 remained slightly over the given limit (Fig. 4). Unstable compost can produce odours, generate harmful compounds and, depending on the plant variety, reduce seed germination and inhibit plant growth (Mathur et al., 1993; Emino & Warman, 2004; Harrison, 2008). Temperature fluctuation in the treatment F2 windrow indicated unstable microbial activity during the composting process, resulting in a higher amount of undecomposed organic matter, as well as a higher respiration rate. Treatment F1, F4 and F5 windrows were very stable; their oxygen consumption was less than 5 mg $O_2$ $g^{-1}$ DM, indicating that composts with longer maturation periods are more stable and safe to use.

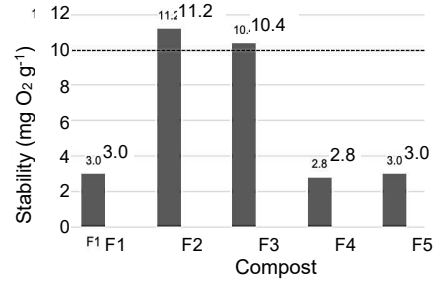


**Figure 4.** Compost stability determined as 4-days oxygen consumption. Values < 10 mg $O_2$ $g^{-1}$ DM indicate stabile compost according to Estonian regulation for sewage sludge compost (Regulation, 2017).

**Quality of composting process**

The effect of treatment, ambient temperature and composting duration on composting temperature was significant ($P < 0.05$) (Table 4).

**Table 4.** Results of the three-way analysis of variance (ANOVA), testing the effect of treatment (TR), ambient temperature (AT), length of the composting duration (TIME), and the interactions between these factors, on composting temperatures

| | Df | Sum of Square | Mean Square | F value | Pr (>F) | |
|---|---|---|---|---|---|---|
| TR | 3 | 1,154,750 | 384,917 | 3,891.97 | $< 2e^{-16}$ | *** |
| TIME | 1 | 1,732,648 | 1,732,648 | 17,519.15 | $< 2\times10^{-16}$ | *** |
| AT | 1 | 27,913 | 27,913 | 282.23 | $< 2e^{-16}$ | *** |
| TR × TIME | 3 | 195,880 | 65,293 | 660.19 | $< 2e^{-16}$ | *** |
| TR × AT | 3 | 41,251 | 13,750 | 139.03 | $< 2e^{-16}$ | *** |
| TIME × AT | 1 | 17,517 | 17,517 | 177.12 | $< 2e^{-16}$ | *** |
| TR × TIME × AT | 3 | 5,178 | 1,726 | 17.45 | $2.66e^{-11}$ | *** |
| Residuals | 11,465 | 1,133,890 | 99 | | | |

Stars indicate significance: *** – 0.001.

**Properties of compost**

There were significant differences in P, K and Mg concentrations between treatments ($\alpha = 0.05$). Treatment F4 and F5 windrows had significantly different nutrient contents of P and Mg, compared to other treatments. Compared to other types of compost (e.g. made from horse manure, sewage sludge and green waste), fish compost contains considerably more P (488–918 mg 100 $g^{-1}$; author's unpublished data; Table 5. This could support the argument that clay minerals bind nutrients together, increasing nutrient content in the final product. It has been shown that fish waste is rich in nutrients such as P and Ca. Fish-derived amendments behave as slow-release N fertilisers, and have high P release capacity (Laos et al., 2000). Therefore, fish compost can be an important source of P based on excavated non- renewable mineral resources.

**Table 5.** Nutrient concentration (mg 100 $g^{-1}$) of fish (F1-F5), horse manure (HM), sewage sludge (SS) and green waste (GW) composts. Different letters indicate different groups according to Tukey *HSD* test ($\alpha = 0.05$)

| Compost | P | K | Ca | Mg |
|---|---|---|---|---|
| F1 | 639$^{b}$ | 791$^{cd}$ | 4,471$^{ab}$ | 703$^{a}$ |
| F2 | 593$^{bc}$ | 1,329$^{bc}$ | 4,626$^{ab}$ | 664$^{a}$ |
| F3 | 488$^{c}$ | 1,676$^{b}$ | 3,166$^{ab}$ | 680$^{a}$ |
| F4 | 802$^{a}$ | 668$^{cd}$ | 2,680$^{ab}$ | 291$^{c}$ |
| F5 | 919$^{a}$ | 1,031$^{cd}$ | 2,927$^{ab}$ | 301$^{c}$ |
| HM | 136$^{d}$ | 2,551$^{a}$ | 2,986$^{ab}$ | 649$^{a}$ |
| SS | 118$^{d}$ | 552$^{d}$ | 1,170$^{b}$ | 518$^{b}$ |
| GW | 115$^{d}$ | 552$^{d}$ | 6,682$^{a}$ | 224$^{c}$ |

Total N concentration in treatment windrows varied from 2.43–2.77%, while total N in alternative composts varied from 0.6–3.74%. Fish composts and horse manure compost had the most similar total N concentrations (Table 5). Brinton & Seekins (1994) have stated previously, that total N concentration of fish compost is 1.29% after 60 days of composting and 1.15% when compost was mature over-winter.

**Table 6.** Total C and N concentration (%) and C:N ratios of fish (F1-F5)-, horse manure (HM)-, sewage sludge (SS)- and green waste (GW) composts

| Compost | C% | N% | C:N |
|---|---|---|---|
| F1 | 30.22 | 2.43 | 12.44 |
| F2 | 33.04 | 2.75 | 12.01 |
| F3 | 35.66 | 2.73 | 13.06 |
| F4 | 27.92 | 2.77 | 10.08 |
| F5 | 27.64 | 2.74 | 10.09 |
| HM | 30.99 | 2.47 | 12.55 |
| SS | 27.02 | 3.74 | 7.22 |
| GW | 13.35 | 0.60 | 22.25 |

One possibility for assessing potential N availability is to observe the C:N ratio of the compost, as it has been shown that available N should be present if the C:N ratio is less than 20:1 (Harrison, 2008). The C:N ratios in various composts were less than 20:1, with the exception of green waste compost (Table 6). Our results are in agreement with the idea that composts made primarily from green waste are nutrient-poor (Harrison, 2008).

## CONCLUSIONS

Fish waste can be processed into nutrient-rich soil amendments via the simple method of composting in windrows, which can be performed near fishing harbours. Fish compost is particularly rich in P and Ca. We suggest mixing fish compost ingredients with a feed mixer or similar mixing equipment, and to use a biofilter with negative aeration in order to reduce odours. Different additives, especially lake sediment with clay minerals, enhances the efficiency of the composting process. The thermophilic

phase was reached in treatment windrows within 1–2 days, and temperatures exceeded 70 °C for at least 1 h, as required by the biofilter EU Commission regulation EU 142/2011 on animal by-products. Our work indicates that nutrient rich compost holds high potential as an organic fertiliser and soil improver, offering the possibility to reduce the use of synthetic fertilisers.

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# II

**Lanno, M.**, Kriipsalu, M., Shanskiy, M., Silm, M., & Kisand, A. (2021). Distribution of Phosphorus Forms Depends on Compost Source Material. Resources, 10 (10), ARTN 102. doi: 10.3390/resources10100102.

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# Distribution of Phosphorus Forms Depends on Compost Source Material

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**Abstract:** Composting is a sustainable method for recovering nutrients from various organic wastes, including food waste. Every input waste has different nutrient contents, in turn, suggesting that every compost has different fertilizer and/or soil improvement values. The phosphorus (P) concentration and relative distribution of P forms is related to the original organic material. The relative distribution of P forms determines how readily plants can absorb P from the compost-amended soil. The aim of this study was to investigate the content and relative share of P forms in composts made from fish waste, sewage sludge, green waste, and horse manure. Six forms of P (labile; bound to reducible metals; bound to non-reducible metals; bound to easily degradable organic material; and bound to calcium) were determined using sequential extraction method. The results indicated that fish waste compost had relatively high proportion of labile P, suggesting good biological availability. In comparison, sewage sludge compost contained the highest overall P concentration per dry weight unit, while labile P constituted only 6% of summary of P forms. The results indicate that the evaluation of composts as alternative P sources in agriculture should rely on the relative distribution of P forms in the compost in addition to the typically recognized value of the total P.

**Keywords:** organic fertilizer; sequential fractionation; fish waste; manure; sewage sludge; green waste; circular economy

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## 1. Introduction

With the growing human population, the need for more sustainable agriculture and food production is increasingly essential. Likewise, as the population grows, the need for fertilizers increases. To ensure the higher crop yield, nitrogen (N) and phosphorus (P) fertilizers are widely used [1].

In general, P is added to the soil with chemical fertilizers. As phosphorite is mined from Earth's crust, our natural P resources become increasingly limited [2,3]. Similarly, significant quantities of P are being lost through the entire cycle of organic wastes. Furthermore, indiscriminate application of chemical fertilizers can exhaust the soil in the long term [4,5]. P run-off can result in environmental pollution and eutrophication in water bodies. To avoid increased P concentrations in streams and lakes, it is important that P added with fertilizers is assimilated by plants [2,4,6].

Alternative P sources are needed to ensure sustainable crop production. To close the P cycle and promote food security and environmental integrity, the European Commission has announced the preparation of an Integrated Management Action Plan (INMAP) with the purpose of reducing the nutrient losses and stimulating the market for recovered nutrients [7]. Options for reducing P losses include the redistribution of P inputs, P recovery and recycling, and redefining P in the food web [8,9]. Organic materials that could potentially be recycled to recover P include manure, wastewater sludge, animal by-products, food waste, and other green wastes [10].

The composting of organic residues is one method of recycling organic materials. Composting can reduce the share of organic waste in landfills and reduce and stabilize the organic material [11]. As composting is an established method of processing organic wastes from various sources, requirements for safety, quality and labelling are established [12].

Compost is a natural fertilizer and/or soil improver that enhances soil chemical and physical parameters and provides essential nutrients, including P, for plant growth [13]. The method of composting can influence the P forms in compost and, in turn, its availability for plants [14,15]. The P concentration in compost depends on the input material as well as the composting process, time, and conditions. The availability of P for plants is determined not only by the amount of P in the compost but also by the P forms. As the nutrient concentration in compost depends on the original organic material, different composts contain different amount of P and different relative distribution of P forms [16,17].

This determines how well plants can absorb P from compost-amended soil and affects the marketing of compost products. Bioavailable P is needed for plant production. Plant roots mainly absorb inorganic P from the soil solution [2,18,19]. As the bioavailability is connected to solubility, water-soluble forms are the most bioavailable, followed by P adsorbed on manganese (Mn) and iron (Fe) oxides, organic P forms, and residual P forms [20]. Often, composts are characterized by their total P content, and no detailed identification of different P forms is required in compost quality manuals, fertilizer regulations, or national end-of-waste regulations [12,21,22].

In the frames of the circular economy, bio-waste is an important resource for organic soil improvers and/or fertilizers. Compost, which is an organic fertilizer composed of different organic materials, has high nutritional value, including P. In general, the total P concentration must be made available according to the requirements for bio-waste management [12]. However, composts of different organic materials have distinct concentrations of total P and P forms [16,23]. We studied the relative share and distribution of P forms in fish waste composts compared with composts from other organic source materials. Based on the P content and relative share of different P forms, we show that composting low-value fish or fish-processing residues is a potentially feasible way to recover P for agricultural use.

## 2. Materials and Method

### *2.1. Preparations of Composts*

Six composts of different source material were used. Experimental composts were prepared from fish waste (FW1-FW3), horse manure (HM), green waste from municipal areas (GW), and sewage sludge (SS) from the regional municipal Wastewater Treatment Plant (WWTP) (Table 1). The WWTP uses $Fe_2(SO_4)_3$ salt PIX 113 (Kemira Oy, Helsinki, Finland) to chemically precipitate P from wastewater. All composts were processed in outdoor conditions in windrows and mixed mechanically with a windrow turner. All windrows were approximately 1.5 m high, 3 m wide, and 12 m long, and covered with semi-permeable geotextile KSV 200 (Compost Systems GmbH, Wels, Austria).

The optimal time–temperature regime depends on the input material. For waste containing animal by-products, such as fish waste and horse manure, the temperature must exceeded 70 °C for at least 1 h, as required by the EU Commission regulation EU 142/2011 [24]. For composting biodegradable waste and sewage sludge, the temperature must exceed 55 °C for 10 days, or 65 °C for 3 days [22,25]. All composts were maintained in accordance with the requirements of these regulations. The duration of active composting was measured as a period when no further significant temperature increase occurred after the mixing of windrows.

**Table 1.** Description of the composts. The share of organic material was calculated using the wet weight. The active composting time (d) indicates the duration in days that composts were in outdoor windrows.

| Treatment Name | Source of Waste | Amendments | Active Composting Time (d) |
|---|---|---|---|
| FW1 | Fish waste (80%) | Un-shredded straw (20%) | 185 |
| FW2 | Fish waste (57%) | Shredded straw (43%), | 70 |
| FW3 | Fish waste (22%) | Shredded straw (22%), peat (22%), inoculum from mature HM (21%) and FW1 compost (13%) | 50 |
| HM | Horse manure (60%) | Straw (40%) | 90 |
| GW | Green waste (100%) | None | >180 |
| SS | Sewage sludge (90%) | Un-shredded straw (10%) | 120 |

### 2.2. Chemical Analysis

Approximately 250 g of average samples were collected from each batch of ready-made compost. One sample contained homogenized compost from three different locations 20–30 cm inside the windrow. The collected samples were dried at 105 °C and ball-milled prior to analysis. Dry matter (DM) and loss of ignition were determined. The total C and N content was analyzed using a VarioMAX CNS analyzer (ELEMENTAR, Langenselbold Germany). Potassium (K), calcium (Ca), and magnesium (Mg) content was determined using the ammonium lactate (AL) method as described by Egner et al. [26]. The compost stability was determined by the four-day cumulative oxygen consumption via OxiTop manometric respirometry system (Xylem Analytics Germany Sales GmbH & Co KG, Weilheim Germany according to the manufacturer's recommendations (Table 2).

**Table 2.** Characterization of composts.

| Treatment | N | C | C/N | K | Ca | Mg | Dry Matter | Loss of Ignition | Stability |
|---|---|---|---|---|---|---|---|---|---|
| | (%) | | | (mg/100g) | | | (%) | | (mg $O_2$/g) |
| FW1 | 2.43 | 30.22 | 12.44 | 790.51 | 4471.04 | 703.31 | 53.80 | 75.40 | 3.00 |
| FW2 | 2.75 | 33.04 | 12.01 | 1329.22 | 4625.55 | 664.00 | 47.94 | 85.78 | 11.20 |
| FW3 | 2.73 | 35.66 | 13.06 | 1675.63 | 3166.32 | 680.48 | 34.79 | 87.05 | 10.40 |
| HM | 2.47 | 30.99 | 12.55 | 2550.90 | 2986.40 | 649.17 | 37.83 | 79.67 | 3.20 |
| GW | 0.60 | 13.35 | 22.25 | 552.08 | 6681.84 | 223.96 | 41.78 | 65.46 | 0.90 |
| SS | 3.74 | 27.02 | 7.22 | 551.81 | 1169.69 | 517.71 | 44.16 | 61.34 | 6.70 |

### 2.3. Determination of P Forms

To assess different P forms, a modification of the determined sequential extraction method was applied [27]. Using this method, six operationally defined P forms were determined (RP—reactive P; and NRP—non-reactive P):

- $NH_4Cl$-RP—"Labile P", immediately available P, which is loosely bound;
- BD-RP P—"Fe-P", bound to reducible metals (Fe, Mn);
- NaOH-RP—"Al-P", phosphates absorbed to metals (Al, $M_xO_y$);
- NaOH-NRP—"Org-P", bound to organic material and colloids, determined as difference between soluble reactive P and total P in NaOH extract; and
- HCl-RP—"Ca-P", bound to calcium (Ca).

From the collected samples, 0.025 g of dried material was analyzed in three replicates (Figure 1). For extraction, 10 mL of 0.1 M $NH_4Cl$ was added to the compost sample in 15 mL Falcon centrifugation tubes and rotated using a Hettich Universal 320 centrifuge (Andreas Hettich GmbH & Co. KG, Germany) for 2 h. After 10 min of centrifugation at 4000 rpm, the supernatant was collected for 2 h. After 10 min of centrifugation at 4000 rpm,

the supernatant was collected for further analysis. We added 10 mL of 0.1 M NaOH to the pellet for a repeated extraction step.

In a third step, 10 mL of 0.11 M bicarbonate/dithionite (BD) solution was added to the pellet and incubated for 1 h at 40 °C, followed by centrifugation, supernatant removal, and rinsing of the remaining sediment pellet with another 5 mL of BD solution. The latter was added to the previously decanted 10 mL of BD extract after centrifugation. In the next step, 10 mL of 0.1 M NaOH was added to the sediment pellet, and the tubes were rotated for 16 h, followed by centrifugation and supernatant collection. In the final extraction step, 10 mL of 0.5 M HCl was added to the pellet, and the tubes were rotated for 24 h.

The soluble reactive P (SRP) in each extract was determined using a spectrophotometer Shimadzu UV-1800 (Shimadzu Corporation, Japan) via the molybdenum blue method [28]. The total P concentration in the NaOH extract was also determined as SRP after persulphate digestion of the NaOH extract at 121 °C for 1 h. Specifically, 2 mL of 5% $K_2S_2O_8$ was added to 10 mL of solution containing 2 mL of NaOH extract, 0.5 mL 0.5 M HCl and 7.6 mL distilled water and autoclaved. If needed, the solution was diluted prior absorbance measurement. The NRP concentration in the NaOH extract was calculated as the difference between the total P and SRP concentration in the extract.

NaOH extract from sewage sludge and fish compost samples contained white haze after persulphate digestion. To remove the haze, the solution was filtered through a 0.45 µm pore size syringe filter Millex-LCR Hydrophilic PTFE (Merck KGaA, Germany) before adding a molybdenum reagent. Based on the concentration of the analyzed P forms, the summary of determined P forms (SumP) was calculated.

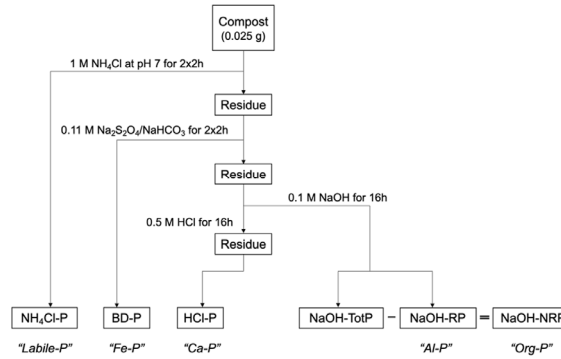


**Figure 1.** Phosphorus (P) fractionation scheme. Labile-P is water soluble and loosely bound P; Fe-P is iron bound P; Ca-P is calcium bound P; Al-P is aluminum bound P; and Org-P is P bound to organic material.

### *2.4. Statistical Analysis*

Statistical analysis was performed in R [29]. The Shapiro–Wilk test was used to confirm normality. One-way ANOVA was used to study the effect of compost type on different P forms. The Tukey HSD post-hoc test, using the package "agricolae", was performed for pairwise comparisons [30].

## 3. Results

Six composts with different organic material each contained a different SumP (Figure 2). SumP varied, on average, from 2.73 g P/kg dry weight (DW) in the GW compost to 16.27 g P/kg DW in the SS compost (Table 3). FW composts ranged from 10.40 g P/kg DW

in the FW3 compost to 13.53 g P/kg DW in the FW2 compost. Statistically, FW 1–3 contained similar SumP to both the SS and HM composts.

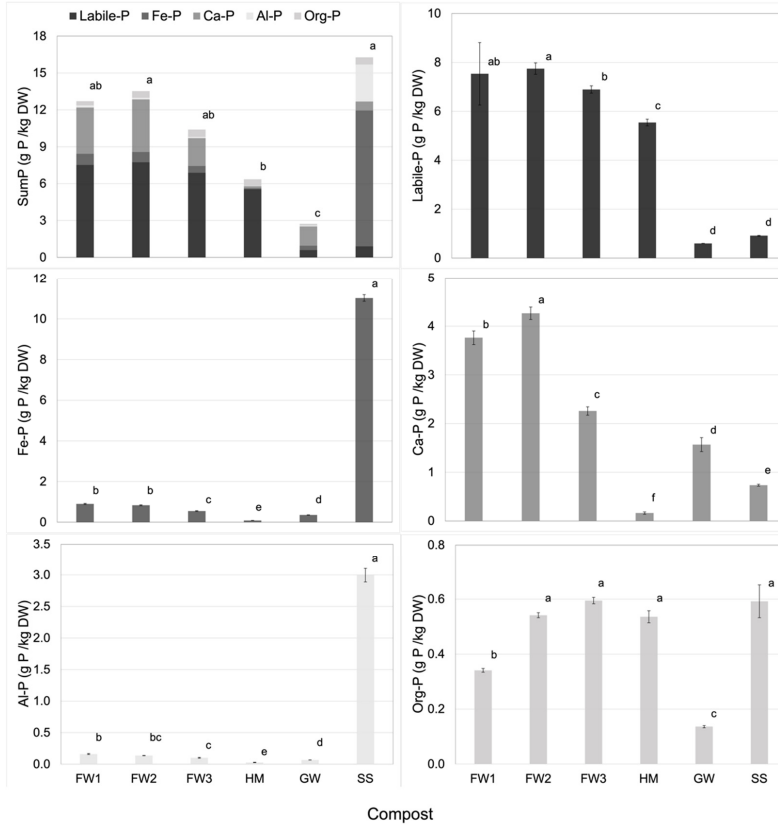


**Figure 2.** Concentrations of different phosphorus (P) forms (g P/kg DW). Labile-P is water soluble and loosely bound P; Fe-P is iron bound P; Ca-P is calcium bound P; Al-P is aluminum bound P; and Org-P is P bound to organic material. Different letters indicate statistically significant differences between groups, according to the Tukey HSD test ($\alpha$ = 0.05).

**Table 3.** Average phosphorus (P) concentrations (g P/kg DW). Labile-P is water soluble and loosely bound P; Fe-P is iron bound P; Ca-P is calcium bound P; Al-P is aluminum bound P; and Org-P is P bound to organic material.

| Compost | Labile-P | Fe-P | Ca-P | Al-P | Org-P | SumP |
|---|---|---|---|---|---|---|
| FW1 | 7.53 | 0.90 | 3.76 | 0.16 | 0.34 | 12.70 |
| FW2 | 7.75 | 0.83 | 4.27 | 0.14 | 0.54 | 13.53 |
| FW3 | 6.90 | 0.55 | 2.26 | 0.10 | 0.59 | 10.40 |
| HM | 5.55 | 0.09 | 0.16 | 0.03 | 0.54 | 6.36 |
| GW | 0.60 | 0.35 | 1.57 | 0.07 | 0.14 | 2.73 |
| SS | 0.92 | 11.03 | 0.74 | 3.00 | 0.59 | 16.27 |

In addition to SumP, the composts varied in concentration of P forms. Concentration of labile-P, the most bioavailable P form, was highest in the FW2 compost (7.75 g P/kg DW), followed by FW1 (7.53 g P/kg DW) and FW3 composts (6.90 g P/kg DW). The lowest concentration of labile-P was in the GW compost (0.92 g P/kg DW). While the SS compost had the highest SumP, its concentration of labile-P was similar to that of the GW compost.

Compared to other composts, the SS compost contained at least 12-times higher concentration of Fe-P (11.03 g P/kg DW). Similar results were observed for Al-P, of which the concentration in most composts did not exceed 0.16 g P/kg DW; while in the SS compost, the Al-P concentration was 3.00 g P/kg DW. This is presumably related to the sewage sludge from external small-size WWTPs, where $Al_2(SO_4)_3$ is used for wastewater precipitation.

The FW compost contained a remarkable amount of apatite, likely related to the Ca-P from the fish bones. The highest concentration of Ca-P was in the FW2 compost (4.27 g P/kg DW), followed by FW1 (3.76 g P/kg DW) and FW3 (2.26 g P/kg DW). As green waste is collected from the area, where limestone bedrock is close to the surface, the GW compost contained a considerable amount of Ca-P (1.57 g P/kg DW). The lowest concentration of Ca-P was observed in the HM compost (0.16 g P/kg DW).

The FW2, FW3, HM, and SS composts contained similar amounts of Org-P, (ranging from 0.54 g P/kg DW in the HM compost to 0.59 g P/kg DW in the FW3 compost).

The relative share of P forms varied according to the organic material of the composts. The highest relative share of labile-P was in the HM compost at 87% of SumP (Figure 3). While the FW composts contained the highest concentration of labile-P per g P/kg DW, the relative share of labile-P varied in composts FW1–3 (57–66%). The SS compost with the highest SumP contained the lowest share of labile-P (6%).

Generally, the relative share of Fe-P and Al-P in various composts were relatively low (Fe-P < 13% and Al-P < 3%), except in the SS compost, where the share of Fe-P was 68%, and Al-P was 18%. The highest share of Ca-P was in the GW compost (57%), followed by FW composts (up to 32%). The SS compost contained 5%, and the HM compost contained 3% of Ca-P. The share of Org-P remained <10% in every compost.

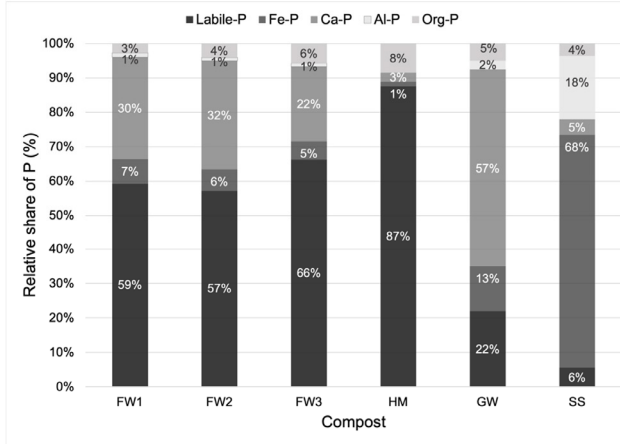


**Figure 3.** Relative share of phosphorus (P) forms (%). Labile-P is water soluble and loosely bound P; Fe-P is iron bound P; Ca-P is calcium bound P; Al-P is aluminum bound P; and Org-P is P bound to organic material.

## 4. Discussion

Ecologically significant P forms of composts were determined in our study. The difference between SumP and total P is referred to as residual P [31]. Since residual P is considered to be a stable P form that does not add to the pool of bioavailable P, it is a less significant P form from the aspect of fertilization [32,33]. In the present study, the compost with the greatest SumP was the SS compost, followed by FW composts, the HM compost, and finally the GW compost.

In the present study, the summarized content of P forms in FW composts was relatively high. The total P content of FW composts in other studies were reported as 2.6 g P/kg DW [34] and 6 g P/kg DW [35]. A compost's P concentration depends on both the relative share of bulking material and the composition of fish waste. The total P concentration in fish and fish waste has been reported to fall into a range of 1–3.5% of dry weight (respectively, 10–35 g P/kg DW) [36,37]. Fish bones, which contain large proportions of hydroxyapatite, can have P concentrations above 120 g P/kg DW [36,38]. Straw, pine bark, sawdust, seaweed, and peat are examples of bulking agents used in fish composts [34,35]. In the present study, straw and peat were the main bulking materials for FW composts, whereas fish waste constituted up to 80% (on a wet weight bases) of the initial contents of the FW composts.

Composts with different organic materials have different concentrations of P forms [16,23]. Fish waste is known to be a good source of P for plant growth [34,36]. In the present study, 7.75 g P/kg DW in the FW2 compost, and over 50% of P in all FW composts, was present in a labile, potentially bioavailable form. FW composts also contained relatively high shares of Ca-P, most likely due to the high presence of hydroxyapatite in fish bones and scales. Ca-P is regarded as a stable P form [19,39]. In another study on fish bones, easily-extractable P was shown to be 4.21 g P/kg DW, while the total P in fish bones was 120 g P/kg DM [40].

Although FW composts contained the highest concentration of labile-P per DW, the relative share of labile-P was highest in the HM compost. As FW and HM composts have high ratios of readily-bioavailable P, they have a high potential to partly replace mineral P fertilizers and, thus, reduce the dependency on P fertilizers derived from phosphorite.

Water soluble P is bioavailable for plants; however, in the case of overuse, it has a high potential to be easily leached from the soil. Therefore, knowledge regarding the distribution of P forms in composts could be valuable when evaluating composts as sources of P fertilizers [23,39].

To reduce the leaching of P from soils, thereby reducing P pollution in water bodies, co-composting wastes with higher proportions of bioavailable P together with the wastes with low concentration of bioavailable P should be considered [16]. Thus, it may be beneficial to co-compost fish waste and green wastes. It has been shown that green waste low in sumP and labile-P concentrations represents a suitable material to compost in combination with other organic materials to enhance the degradation of organic matter, humification, and nutrient content [41].

Studies of P forms in organic source materials have revealed low portions of labile-P but high total P in sewage sludge, which is in accordance with the present study. Sewage sludge total P content was reported to be higher than 30 g P/kg DM; however, after mixing with soil, only 1–2% of the total P was in labile form [31]. In the present study, the SS compost contained the highest overall P concentration per DW; however, a low concentration of it was in a potentially bioavailable form. Instead, the majority of it was bound to Fe and Al compounds. Nevertheless, depending on the environmental conditions, P added to soil can alter the composition of P forms and its bioavailability for plants [19,39]. Related to the dynamic equilibrium between P pools, P is continuously released from other fractions to the bioavailable form [19].

It has been suggested that P bound to Al and Fe may become mobilized when labile-P pools are depleted from the soil [42]. It was shown that, depending on the soil pH, Al-P can have higher bioavailability than Fe-P or Ca-P, and therefore Al-P is considered as a potentially bioavailable form [39,43]. Fe-P is a highly stable P form in oxic conditions; however, it can become unstable at certain times of the year, when anaerobic soil conditions occur or when lower pH conditions catalyze Fe reduction, thus, resulting in the dissolution of both Fe and Fe-P [39,43]. As the SS compost in the present study contained a relatively high concentration of P in fixed form (18% Al-P and 68% Fe-P), we recommend application of biological rather than chemical P removal in WWTPs when considering further use of sewage sludge as fertilizer.

SS composts are subject to national wastewater regulations and may exceed the limit values of heavy metals or other safety and quality indicators. Therefore, the requirements and limits for the use of SS composts have been stated [44]. However, the potential of sewage sludge for agricultural use has been discussed, and, with potentially high bioavailable P content, it may represent an alternative to chemical fertilizer for crops of moderate demand [45–47].

As composts vary in nutrient content, it may be beneficial to combine both organic and mineral fertilizers, as their integration has been reported to improve P solubilization, as well as minimize P precipitation in soil and, thus, has helped in increasing the P availability for crops [48].

## 5. Conclusions

Composts containing different organic source materials—fish waste, horse manure, green waste, and sewage sludge—showed significant differences in the content and relative share of phosphorus forms.

Green waste (GW; from leaves, branches, and plant residues) and horse manure (HM) composts are widely used. However, GW compost is low in P content, and a relatively low percent of it is in labile-P form. HM compost, which is highly valued in agriculture, is rich in P content, and the majority of P was in labile-P form. This should be considered when calculating the compost application rate.

Sewage sludge (SS) compost contained the highest concentration of P content. However, the majority of it was bound to Fe or Al, reducing the availability for plant uptake. P in sewage sludge is from biological or chemical P-removal from wastewater. There-

fore, the P concentration in sewage sludge is related to the P removal efficiency during wastewater treatment, as well as whether the P removal is biological or Fe- or Al-based. To ensure better P bioavailability for plants, we recommend biological P removal during wastewater treatment.

Fish waste (FW) compost is not widely used although it is very rich in P content, and a relatively high concentration of this is in labile-P form. Therefore, it could be suitable for crops that require available P immediately. Food waste that has equal compost qualities as fish waste should be re-assessed as a compost source and returned to agricultural production accordingly.

Composts represent valuable sources of recovered nutrients, including P, and should be considered as an alternative to mineral P fertilization, allowing recycling of the P present in organic wastes back into soils. The evaluation of different composts as a source of P fertilizer should consider the relative share and potential bioavailability of different P forms present in the composts.

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# III

**Lanno, M.**, Klavins, M., Purmalis, O., Shanskiy, M., Kisand, A., & Kriipsalu, M. (2022). Properties of Humic Substances in Composts Comprised of Different Organic Source Material. Agriculture, 12(11), 1797. https://doi.org/10.3390/agriculture12111797

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# Properties of Humic Substances in Composts Comprised of Different Organic Source Material

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**Abstract:** Reusing organic waste as fertilizer is one method to reduce the use of mineral fertilizers and minimize waste disposal in landfills. Regulations have been enacted for the processing of organic waste and for recycling end products, but the humic content of organic fertilizers has been neglected. We studied seven composts with different organic input materials and technologies. Humic substances (HSs) were detected in all composts. The total organic carbon in the HSs constituted 8.7 ± 0.1% (SD)–27.0 ± 0.2% of the compost dry matter. Spectral differences between the studied samples in FTIR spectroscopy could be observed at 1700–1000 $cm^{-1}$, indicating differences in compost precursor material. The EEM peak, associated with humic acids (HAs), was high in composts containing animal by-products (e.g., fish waste, horse manure, and kitchen biowaste). Kitchen biowaste, also when processed by *Hermetia illucens* larvae and vermicompost, exhibited slower organic material transformation with low humic acid/fulvic acid ratios (<1.60). The results show the importance of source material origin and amendments, which influence the composting process and final products. Our study emphasizes the role of humic substances in the comprehensive evaluation of composts. To maximize the added value of composts, marketing strategies should consider determining the share of humic substances besides the content of organic matter and nutrients.

**Keywords:** humic acid; fulvic acid; fish waste; horse manure; sewage sludge; *Hermetia illucens* frass; green waste; kitchen biowaste

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## 1. Introduction

With rapid urbanization and population growth, global annual waste is expected to increase by 3.4 billion tons by 2050 [1]. It is estimated that, across the European Union (EU), up to 138 million tons of bio-waste is generated annually, of which only 60 million tons is recycled through composting and anaerobic digestion [2]. About 120–130 tons of bio-waste are generated in Estonia annually, of which 30% is recycled. Unsegregated bio-waste is incinerated or disposed in a landfill [3]. Managing this waste to minimize environmental impacts is increasingly important. Waste disposal, especially organic waste disposal in landfills, causes various environmental impacts, including landfill gases, contributing to air pollution and climate warming and leachate contaminating groundwater [4]. Within the framework of a circular economy, EU waste policies (directives 2018/850 and 2018/851) aim to reduce the landfilling of municipal waste to 10% of current levels by 2035 and to increase the re-use and recycling of municipal waste up to 65% [5,6]. This ambitious policy objective is only achievable by implementing organic-waste-management strategies.

The dependence on mineral fertilizers for agriculture can threaten food security if they become less available. Limited reserves of mineral fertilizer raw materials and changes in supply chains due to various environmental, political, and epidemiological crises all affect the price and availability of mineral fertilizers [7,8]. At the same time, the EU is conducting the Green Deal, which aims to reduce greenhouse gas pollution and slow down climate

change [8]. To increase self-sufficiency and reduce environmental impacts, the supply of alternative fertilizers could be increased at the local level.

It was stated within EU regulation 2019/1009 that organic fertilizer shall contain organic carbon ($C_{org}$) and nutrients of a solely biological origin [9]. Composting is a common biological treatment option, suitable for processing organic waste from various sources. Mature compost is a stable and nutrient-rich, humus-like product, representing a valuable source of recovered nutrients. The concentration of nutrients is determined by input materials. The determination of total nutrient contents for fertilizing purposes is regulated, but results do not describe the proportion of nutrients that are readily available to plants [10,11]. The quality of mature compost is described as a combination of two criteria. First, the precautionary criteria (hygiene parameters, impurities, weed seeds, and inorganic pollutants, such as heavy metals) set limits to prevent environmental pollution and the spread of diseases [9]. Second, quality criteria provide information about fertilizing properties, such as nitrogen (N), phosphorus (P), potassium (K) and micronutrients, organic matter content, salinity, and pH [12,13].

Composts tend to be compared to mineral fertilizers, despite having more complex compositions [14,15]. A distinctive feature of compost is the presence of natural organic matter, reflecting a wide range of environmental and biowaste degradation processes. In Europe, an estimated 45% of soils are low in organic matter content, which lowers soil productivity and increases the risk of soil degradation [16]. With climate change, it is estimated that global air temperatures will increase by 2 °C by 2100 compared to pre-industrial levels, which will change environmental conditions and agricultural production [8,17]. Soils with a higher organic matter content are more resistant to drought and climate change [18]. Organic matter is a nutrient reservoir and can retain nutrients in a plant-available form. Compost can also help restore humus in degraded soils [14,18,19]. While compost has various quality standards regarding fertilizing properties and has limits to prevent environmental pollution and the spread of diseases, it is often overlooked that compost adds humic substances (HSs) to the soil [9,20]. HSs, including humic acids (HAs), fulvic acids (FAs), and humin, are part of the soil organic matter (SOM) that forms humus [21]. The formation of fertile soil layers rich in humic substances can take decades in nature, but it takes 6–12 months to produce a humic-acid-like substance by composting [22,23]. HSs are important in soil restoration processes and can be used to promote nutrient uptake, increase soil porosity, enhance nutrient preservation and water-holding capacity, and reduce the abundance of pathogens [24,25]. HS concentration in the compost is correlated with compost maturity and provides added value to the waste material [26,27]. Therefore, the benefits of composts are described by nutrient and HS contents. These natural biostimulants can be an important marketing tool for composts, which are primarily used in agriculture.

The aim of this study is to compare various composts to quantify HSs and determine whether HS content depends on input materials (manure, sewage sludge, vermicompost, fish, food, yard waste, and black soldier frass) or the selected treatment. These results will draw attention to an important feature of compost that has been underappreciated in compost evaluation and marketing processes.

## 2. Materials and Methods

### *2.1. Selection of Composts*

Seven composts of different source materials were investigated. Composts were produced from low-valued fish (C1), horse manure (C2), green waste (e.g., branches, leaves, plant residues) (C3), sewage sludge (C4), and kitchen biowaste (C5). In addition, *Hermetia illucens* frass (C6) and vermicompost (C7) were selected. All composts were prepared in Estonia by different manufacturers or by the authors. Four composts (C2–C5) were industrially produced and commercially marketed. Composts C3, C4, and C5 were certified according to the Estonian end-of-waste regulation [28], while certification was not required for C2 because manures are not classified as waste. Composts C1, C6, and C7 were experimental (Table 1), and compost amendments and composting technologies were

determined by the manufacturer. Straw is a dry and widely available bulking material in Estonia; therefore, it is well-suited for composting wet waste, such as sludge. Shredded wood, another common bulking material, and straw increase porosity, improve air circulation, and adjust C/N ratios (20–30)/1.

**Table 1.** Characterization of composts. The active period (d) indicates the duration that composts C1–C5 were in outdoor windrows, and for C6–C7, the feeding period of *H. illucens* larvae or *E. fetida*, respectively.

| Compost | Target Waste | Amendments | Scale | Certified Quality Regulations | Active Period (d) | Composting Site Location |
|---|---|---|---|---|---|---|
| C1 | Fish waste | Straw, peat, inoculum from previous composts | Industrial | No | 185 | 58°28′41.08″ N 24°49′28.66″ E |
| C2 * | Horse Manure | Straw | Industrial | Yes | 90 | 58°28′41.08″ N 24°49′28.66″ E |
| C3 * | Sewage sludge | Straw | Industrial | Yes | 120 | 58°13′37.89″ N 26°23′10.17″ E |
| C4 * | Green waste from municipal areas | None | Industrial | Yes | >180 | 59°28′14.63″ N 24°54′43.97″ E |
| C5 * | Kitchen Biowaste | Shredded wood | Industrial | Yes | 130 | 59°27′36.08″ N 25°5′17.18″ E |
| C6 | Cafeteria and grocery store biowaste | None | Pilot | No | 51 | 58°23′30.76″ N 26°41′40.43″ E |
| C7 | Kitchen biowaste | Straw, paper, green waste, biochar | Pilot | No | 365 | 58°23′30.76″ N 26°41′40.43″ E |

* Commercially produced compost.

Composts C1–C5 were processed on an industrial scale in outdoor windrows and mixed mechanically with a Backhus 16.30 windrow turner (Eggersmann Anlagenbau GmbH, Bad Oeynhausen, Germany) or an CMC ST 300 windrow turner (Composts Systems GmbH, Wels, Austria). All windrows were approximately 1.5 m high, 3 m wide, and 12 m long and were covered with semi-permeable geotextile KSV 200 (Compost Systems GmbH, Wels, Austria). All composts were maintained in accordance with national biowaste recycling regulations [28,29]. For waste containing animal by-products (ABPRs), such as fish waste and horse manure, the temperature exceeded 70 °C for at least 1 h, as required by EU regulations (EU 142/2011) [10]. To compost biodegradable waste, including kitchen waste and sewage sludge, the temperature had to exceed 55 °C for 10 d or 65 °C for at least 3 d [12,13]. The active composting period was considered complete when the temperature dropped to an ambient level, and after mixing the windrows, no significant temperature increase occurred.

Compost C1 was an experimental treatment made by the authors at an industrial scale. For C1, the targeted waste material, measured as wet weight, was fish by-catch (ABPRs, 22%) amended with structural materials, wheat straw (22%), peat (22%), and inoculated with composts from similar previous batches (34%). Peat and mature compost, which have a small particle size, also served as a binder for fish material [30]. Compost C2 was composed of horse manure (ABPRs, 60%) amended with straw (40%). Compost C3 consisted of municipal sewage sludge (WWTP with a load of 16.733 PE) amended with straw. The ratio of sludge to straw was 1:1 by volume, resulting in a 9:1 ratio by weight. Compost C4 was composed of biowaste from urban green areas. Compost C5 was made of source-separated household biowaste amended with shredded wood (1:1 by volume) and 6% of category III ABPRs from the chicken industry. Treatments C2–C5 were compatible with national compost quality regulations [28].

Treatments C6 and C7 were prepared on a pilot scale. For C6, *H. illucens* larvae were fed for 5l d, with the frass in contact with fresh feed. The frass was sieved off every two weeks

during the cycle. Feed consisted of approximately 150 L of raw vegetable and fruit waste. To ensure a suitable moisture content, hot-pressed rapeseed cake, rye/wheat bread, and 2 L of canola oil were used. In addition to vegetable and fruit waste, approximately 90 L of catering waste was used.

For treatment C7, *Eisenia fetida* were fed in quadruplicate three-liter mesocosms for 136 d. In the beginning of the experiment, 20 earthworms were applied in each mesocosm (average mass 4.12 g). The experiment was conducted in a temperature-controlled room at 22 °C. Moisture was controlled weekly using the hand-held W.E.T. Sensor Kit (Delta-T Devices, Cambridge, England). Vermicompost moisture was kept in a range of 75–80%, as recommended by Khan et al. [31]. The organic feed was mixed with 50 g of biochar (Biolan Baltic OÜ, Lavassaare, Estonia), with properties described by Escuer, 2021 [32]. Biochar was added to increase reproduction and create favorable conditions for *E. fetida*, while also enhancing microbial biodegradation and the composting process [31,33]. For amendments, milled straw, garden and kitchen green waste, and shredded paper were used. Feed calculations assumed that individual *E. fetida* consume half of its body mass per day. Later, feeding was adjusted based on earthworm performance in the mesocosms. For the first feeding, 200 g of feed with 50 g of biochar was applied.

### 2.2. Chemical Analyses

Approximately 250 g of mature compost from three random locations were collected and homogenized from each treatment. For treatments C1–C5, the compost samples were collected 20–30 cm inside the windrow. For treatment C6, *H. illucens* frass was sieved and homogenized before sampling. For treatment C7, subsamples were taken from four replicate mesocosms and mixed to obtain one homogenous sample per mesocosm. Subsamples were collected based on volume, and earthworms and cocoons were separated by hand.

Samples were dried at 105 °C and ball-milled prior to analysis. Elemental analysis (C, N) was conducted using a VarioMAX CNS analyzer (ELEMENTAR, Langenselbold Germany). Potassium (K), calcium (Ca), magnesium (Mg) contents, and phosphorus (P) were determined using the ammonium acetate–lactate (AL) method as described by Egner et al. [34].

Lipids were extracted using 10 g of dried compost sample and 10 mL of $CHCl_3$. The extract was filtered after 24 h through filter paper into a pre-weighed Petri dish. The air-dried lipids were weighed, and their proportion in the compost was calculated.

### 2.3. Extraction of Humic and Fulvic Acids

For HA extraction, 5 g compost and 50 mL 2% NaOH solution were combined and stirred for 4 h. The suspension was filtered and acidified with 6M HCl under constant stirring until pH = 2 was reached, HA precipitated, and only FA remained in the solution. Freeze-dried HA were used to prepare HA solution (50 mg/L) using 0.1 NaOH [35].

Characterization of isolated humic and fulvic acids were conducted as suggested by Klavins and Purmalis [36]. The ratio of HA/FA was calculated the using total organic carbon (TOC) values in HA and FA solutions. TOC analyses were performed using a Shimadzu TOC-V CSN total carbon analyzer (Shimadzu Corporation, Kyoto, Japan), operating at 720 °C.

### 2.4. UV–Vis Absorption Spectra of HA

The absorbance of solubilized HA (50 mg/L) in 0.1 M NaOH was measured using a UV–Vis spectrophotometer Shimadzu UV-1800 (Shimadzu Corporation, Kyoto, Japan), with a 1 cm quartz cuvette and a wavelength range of 230–670 nm. Absorbance ratios, $E_2/E_3$, were calculated using UV–Vis absorption at 250 nm and 365 nm, and $E_4/E_6$ was calculated from the UV–Vis absorption at 465 nm and 665 nm [37,38].

### 2.5. Fourier-Transform Infrared Absorption Spectra (FTIR) of HA

To obtain the spectra, 3 mg of HA sample and 200 mg of KBr powder were mixed together, and 30 mg of the mixture was weighed and compressed into a tablet. The FTIR spectra were obtained using a Fourier-transform infrared spectrophotometer Shi-

madzu IR-Tracer 100 (Shimadzu Corporation, Kyoto, Japan), at an infrared spectrum of 400 to 4000 $cm^{-1}$, with a resolution 4 $cm^{-1}$, and by taking 10 scans.

### *2.6. Excitation Emission Matrix (EEM) Spectroscopy of HA and FA*

Extracted HA and FA samples were analyzed using fluorescence spectroscopy TL spectrometer HORIBA Aqualog (Horiba Ltd., Kyoto, Japan). The EEM spectra were measured at an emission wavelength range of 250–600 nm and an excitation wavelength range of 250–600 nm scanning with 5 nm increments. The obtained spectra were evaluated with PARAFAC analysis using MATLAB R2014 a v. 5.3.0.532 software with the DOMFluor and drEEM toolboxes [39].

### *2.7. Statistical Analysis*

Statistical analysis was performed in R [40]. The results of elemental composition, TOC, and lipids results were presented as mean values of triplicate measurements and compared using ANOVA with a post-hoc Tukey's HSD test [41].

## 3. Results

The nutrient content and pH of the composts is presented in Table 2. Composts C3 and C6 had a remarkably high P concentration, and C5 showed the lowest P and highest N concentration, concurrently. Compost C6 had the lowest Ca and Mg content, while both of these nutrients were high in C2 and C4. The pH of the treatments ranged from 5.77 to 8.10.

**Table 2.** Elemental composition and pH of the composts. Mean ± standard deviation (SD) of triplicate measurements compared using ANOVA with post-hoc Tukey's HSD test.

| Compost | N | C | C/N | Ca | K | Mg | P | pH |
|---|---|---|---|---|---|---|---|---|
| | % | | ratio | μg/g | | | | |
| C1 | 4.3 ± 0.1 | 65.3 ± 0.3 | 15.19 | 493 ± 8 | 1910 ± 25 | 80 ± 5 | 1469 ± 18 | 5.77 |
| C2 | 4.5 ± 0.1 | 62.7 ± 0.3 | 13.93 | 1472 ± 20 | 4700 ± 30 | 234 ± 5 | 1122 ± 20 | 7.40 |
| C3 | 5.7 ± 01. | 60.5 ± 0.3 | 10.61 | 546 ± 9 | 791 ± 12 | 91 ± 5 | 2782 ± 20 | 6.29 |
| C4 | 3.8 ± 0.1 | 63.2 ± 0.3 | 16.63 | 1224 ± 12 | 4164 ± 30 | 145 ± 5 | 796 ± 12 | 7.80 |
| C5 | 8.6 ± 0.1 | 61.9 ± 0.3 | 7.20 | 947 ± 10 | 6412 ± 35 | 58 ± 5 | 120 ± 8 | 6.85 |
| C6 | 7.9 ± 0.1 | 60.5 ± 0.3 | 7.66 | <32 | 114 ± 8 | <3 | 2432 ± 20 | 8.10 |
| C7 | 6.0 ± 0.1 | 62.8 ± 0.3 | 10.47 | 759 ± 8 | 578 ± 10 | 120 ± 5 | 839 ± 12 | 7.69 |

The humification of organic material can be estimated from the HA/FA ratio (Table 3). A higher HA/FA ratio corresponded to higher humification rates of the organic material. The highest HA/FA ratios were measured in treatments C2 (7.88) and C4 (7.82), whereas the lowest HA/FA ratios were observed in treatments C6 (1.46) and C5 (1.42). Treatments C6 and C5 had the highest concentrations of lipids (2.1% and 4.2%, respectively).

**Table 3.** Characterization of HSs in composts. TOC = total organic carbon in humic substances. TOC and lipids are presented as % of compost dry matter. Mean ± standard deviation (SD) of triplicate measurements compared using ANOVA with post-hoc Tukey's HSD test. Connecting letters represent significantly different measurements.

| Compost | TOC | HA/FA | $E_2/E_3$ | $E_4/E_6$ | Lipids |
|---|---|---|---|---|---|
| | % | | | | % |
| C1 | 12.0 ± 0.2 $^{a}$ | 5.70 | 2.65 | 7.78 | 1.5 ± 0.1 $^{b}$ |
| C2 | 27.0 ± 0.2 $^{c}$ | 7.88 | 2.57 | 6.95 | 1.5 ± 0.1 $^{b}$ |
| C3 | 25.7 ± 0.2 $^{c}$ | 4.73 | 2.49 | 8.33 | 1.5 ± 0.1 $^{b}$ |
| C4 | 18.0 ± 0.2 $^{ab}$ | 7.82 | 2.56 | 7.34 | 1.7 ± 0.1 $^{b}$ |
| C5 | 15.3 ± 0.2 $^{a}$ | 1.42 | 2.38 | 6.88 | 4.2 ± 0.1 $^{a}$ |
| C6 | 17.1 ± 0.2 $^{a}$ | 1.46 | 2.66 | 6.40 | 2.1 ± 0.1 $^{b}$ |
| C7 | 8.7 ± 0.1 $^{d}$ | 1.60 | 2.61 | 8.59 | 1.4 ± 0.1 $^{b}$ |

The spectral ratio $E_4/E_6$ shows the overall aromaticity and condensation of the material and can characterize the size of molecules (Table 3) [37]. $E_4/E_6$ depends on the source material. The smallest $E_4/E_6$ ratio, indicating the greatest organic matter polymerization, was observed in compost C6 (6.40), followed by C5 (6.88) and C2 (6.95). The lowest aromaticity was observed in compost C7 (8.59). $E_2/E_3$ describes not only aromaticity, but also the degradation of original material, including the degradation of phenols [37]. When $E_2/E_3$ is < 3.5, the content of HA is higher than that of FA [42]. For all composts, the ratio varied in the range 2.38–2.66.

The UV–Vis spectra of HA (Figure 1) monotonically decreased with the increasing wavelength. The absorption peaks of UV–Vis spectra between 255 and 290 nm are typical for aromatic or unsaturated compounds, such as quinones and ketones [36]. These spectra were visible for C1, C2, and C3. Composts C4, C7, and C6 had small shoulders of spectra, and C5 did not exhibit a shoulder of spectra.

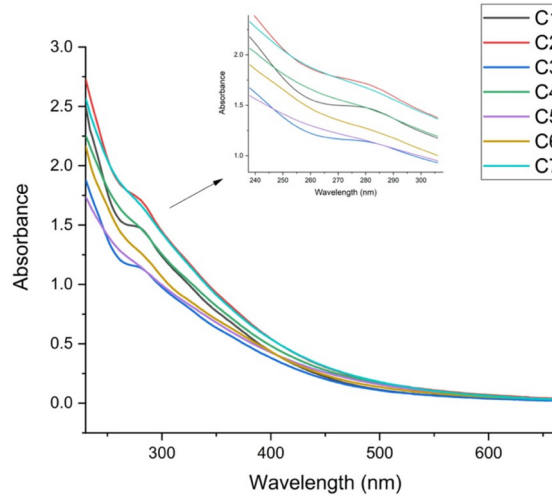


**Figure 1.** UV–Vis spectra. Compost labels according to the target waste: C1 fish waste; C2 horse manure; C3 sewage sludge; C4 green waste; C5 kitchen biowaste; C6 *H. illucens* frass; and C7 vermicompost.

The FTIR spectra of the analyzed samples had a similar pattern, showing the similarity of the obtained HSs and absorption at 3600–3000 $cm^{-1}$, representing -OH groups (Figure 2). Methyl groups in aliphatic compounds were visible at 2920 $cm^{-1}$ and were similar in all samples [43]. At 1700 $cm^{-1}$, the spectra showed the absorption of C=O bonds in carboxyl groups, aldehydes, ketones, and esters, with higher rates in samples C3, C4, C5, and C7. The region at 1637–1610 $cm^{-1}$ shows significantly less signal for C7 and moderately less for C2. Different from other composts, C5 had a small absorption in C-N and N-H bonds in amides, and C7 had none. The region at 1470–1370 $cm^{-1}$ represents carbonyl acids and phenols, and there were visible differences for samples C7 and C5, where the presence of those bands was low. Carboxylic groups and ethers were present in all compost HA samples, with slightly higher amounts for C1 and C4.

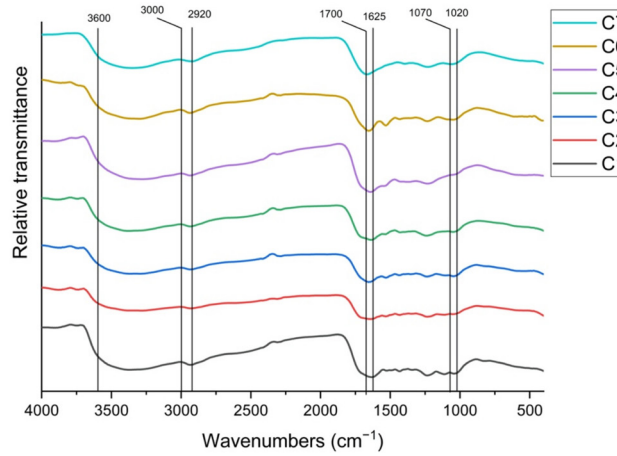


**Figure 2.** Fourier-transform infrared (FTIR) spectra of humic acids from compost samples. Compost labels according to the target waste: C1 fish waste; C2 horse manure; C3 sewage sludge; C4 green waste; C5 kitchen biowaste; C6 *H. illucens* frass; and C7 vermicompost.

Common, and at the same time similar for all samples, was the presence of C-O in carboxyl groups and ethers. The region at 1070–1020 cm$^{-1}$ describes the C-O bonds in polysaccharides, with noticeably less in the C2 and C5 samples. The presence of detected structural features describes the condensation and conjugation of organic material during composting processes.

The compost EEM fluorescence spectra for FA and HA are shown in Figures 3 and 4. All samples exhibited one FA peak at Ex/Em 340–350/440–450 nm, which is related to the FA visible area and associated with polysaccharides. A very small peak was present at Ex/Em 420–450/520–550 nm, representing condensed aromatic structures [44,45].

Other fractions of humic material, such as HAs, have different compositions and dominant peaks in the EEM. Common peaks vary only by dominant peak and relative height for HA, and they include Ex/Em 350–380/470–510, 430/520, and 460–470/540–560 nm. The peak Ex/Em 350–380/470–510 nm is associated with HA-like substances, which form during the humification of composting organic matter [46]. This peak dominated in samples C1, C2, and C5 and was the second largest peak in samples C4 and C6. Similar to FA, the EEM for HA was Ex/Em 430/520 nm peaks, associated with the degradation and condensation of organic material. Ex/Em 460–470/540–560 nm dominated in samples C4, C5, and C7, which probably indicates the presence of lignin from structural amendments (shredded wood, straw, paper). Treatment C6 differed from other samples, with the core of HA peaking at Ex/Em 370–410/610–630 nm. The origin of these compounds could be fresh plants and fruits added to compost and characterized by low degradation rates and chlorophyll [47].

Figure 5 illustrates the comparative evaluation of studied composts, considering both nutrient and HS indicators. On the PCA biplot, composts C1, C5, and C7 group together, mainly due to low HSs, while C3 shows elevated HSs and P, and C6 is rich in P and N with average HSs.

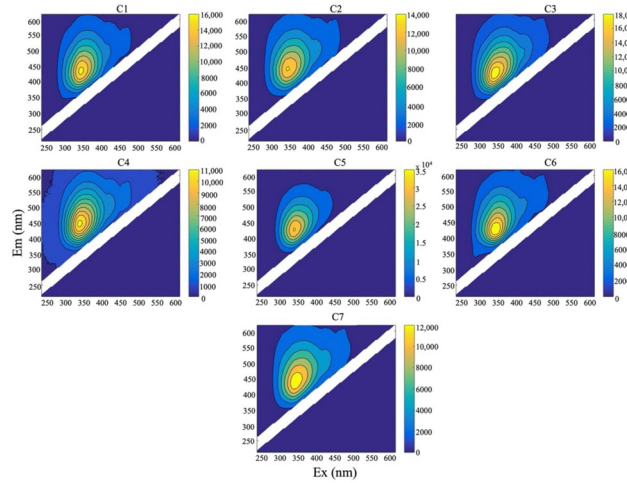


**Figure 3.** Composition of fulvic acids (FAs) for different treatments. Compost labels according to the target waste: (**C1**) fish waste; (**C2**) horse manure; (**C3**) sewage sludge; (**C4**) green waste; (**C5**) kitchen biowaste; (**C6**) *H. illucens* frass; and (**C7**) vermicompost. The color scale is presented in relative units.

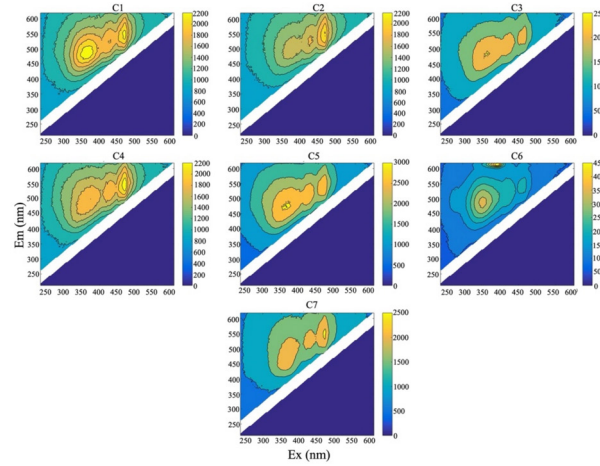


**Figure 4.** Composition of humic acids (HAs) of different treatments. Compost labels according to the target waste: (**C1**) fish waste; (**C2**) horse manure; (**C3**) sewage sludge; (**C4**) green waste; (**C5**) kitchen biowaste; (**C6**) *H. illucens* frass; and (**C7**) vermicompost. The color scale is presented in relative units.

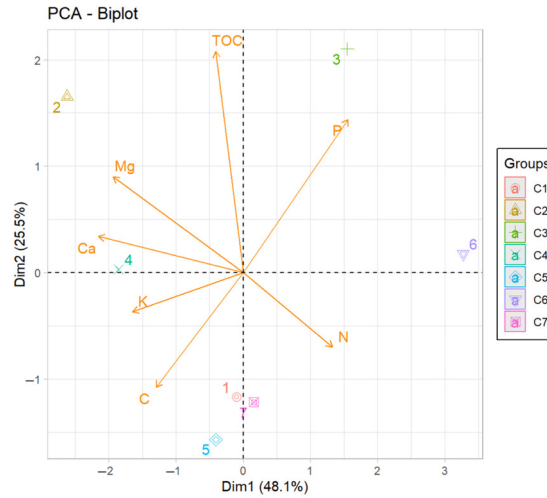


**Figure 5.** Principal component analysis (PCA) of composts based on nutrient and humic substances' (HSs) TOC concentrations. Axis 1 explains 48.1% of the variance, and axis 2 explains an additional 25.5% of the variance. Compost labels according to the target waste: C1 fish waste; C2 horse manure; C3 sewage sludge; C4 green waste; C5 kitchen biowaste; C6 *H. illucens* frass; and C7 vermicompost.

## 4. Discussion

The selected composts varied in input material, selection of treatment, and the active composting time (d). These features are known to affect the concentration and type of HSs in the final product [25,48,49].

In the treatment of organic waste, the concentration of humus-like substances and the degree of humification of hydrophilic components increases, and the structure of the substance becomes more stable [50]. HSs are biostimulants that enhance nutrition efficiency, abiotic stress tolerance, and/or crop quality traits [9,51]. All compost treatments in the present study contained HSs, including HA and FA. While all treatments presented an FA peak for polysaccharides, they differed more relative to the HA peak, showing that input materials directly influence the core structure of HA in mature compost. As shown previously, various input materials contain different amounts of basic components that form HSs [25,50]. Organic waste, rich in polyphenols, amino acids, and reducing sugars can promote overall HS production [52].

During composting, the concentration of FAs decreased, whereas the concentration of HAs increases. HAs are generated in the last stage of composting; thus, an increased concentration of HAs indicates a higher maturity of the compost. Compost is considered mature if the HA/FA ratio is higher than 1 [15,25,53,54], and the HA/FA ratio is also affected by the type of organic material. For example, the lowest HA/FA was in treatment C5, which contained a high quantity of food waste. Amino acids and sugars, e.g., polysaccharides, break down easily and are first to form FA, and then HA [55]. The highest HA/FA ratio was in treatment C2. After fermentation in animal guts, horse manure contained a high proportion of lignin from hay and grass, aromatic compounds, and older material. Lignin and aromatic compound remnants could be the core of the initial HA, and less FAs are formed [23,26].

The chemical structures of HSs were similar in the compost treatments. As shown previously, the UV–Vis spectra of hydrophilic components decreased monotonically, and the observed spectra were typical of aromatic or unsaturated compounds, such as the conjugation of quinones and ketones [50,56].

Treatments C1, C2, and C5, which contained different ABPRs (e.g., fish waste, horse manure, chicken body parts), had a dominant HA peak, reflecting the HA-like substances formed during humification. ABPRs are rich in nutrients and add microorganisms to the composting mixture. In addition to the source materials, the content of HSs depends on the amounts of nutrients (e.g., C, N) and the presence of microorganisms [57]. Humification is more intense and achieved more quickly if composting piles are inoculated with microorganisms. Composts inoculated with microorganisms stimulated the degradation of hemicellulose, cellulose, and lignin by 28%, 21%, and 25%, respectively [58]. The greater the amount of amino acids and reducing sugars produced during degradation, the higher the positive effect on HS synthesis [52].

Treatments C4, C5, and C7 had a dominant peak in the area representing lignin-like structures. These treatments contained large quantities of green waste (e.g., branches, leaves), shredded wood, straw, and paper, which are rich in cellulose and lignin. Selected bulking material affects the composting duration and product quality, as material rich in cellulose and lignin can influence the humification process [25]. These kind of materials decompose more slowly; therefore, they can slow down composting processes and humification [59].

Treatment C6 differed from other composts made from more traditional composting technologies. C6 contained frass from *H. illucens* larvae activity, as well as from chitin and food residues during feeding, and the core structure of HA differed compared to other treatments. Although *H. illucens* larvae frass can be used as an organic fertilizer, the fresh frass might have contained phytotoxins, which decrease plant growth [60]. To increase the concentration of HA and the stability of the frass, it is suggested to post-compost the frass [60,61].

It has been shown, that *H. illucens* treatment can increase the HA/FA ratio and improve humification [62]. Compared to other treatments, C6 had the lowest $E_4/E_6$ ratio, indicating a more advanced humification. However, the HA/FA ratio was quite low, reflecting a high FA concentration. *H. illucens* can also suppress pathogens and is therefore suitable for processing manure in addition to food waste [60,63].

The highest aromaticity was in compost C7, indicating that by vermicomposting, HA is a more aggregated macromolecule with a more incomplete humification processes [64]. However, it has been proposed that there is no direct relationship between aromaticity and humification, and vermicompost can be used as a soil improver [50].

The quality of composts is reflected by the content of both HSs and nutrients. Although some composts may have a lower nutrient concentration, they may still be a valuable source of HSs. The total value of compost is thus reflected by the nutrient content, the disclosure of which is currently required by regulations (Appendix A) [9,13]. Depending on the main purpose of compost application, the effects of added nutrients or added HSs can be the primary advantage of using compost. Including HS data for composts yields a better comprehensive evaluation. Composts C2 and C4 were grouped together on the PCA bioplot, considering only their nutrient content, but elevated HSs in compost C2 signified an advantage over C4 (Figure 5). Composts with a high proportion of HSs, but a relatively low nutrient content, can provide a maximum fertilizing effect when used together with mineral fertilizers. Thus, soil health and nutrient content are ensured to improve crop yield [65,66].

Although different treatments varied in HS concentrations, both HA and FA act as biostimulants and promote plant growth [67,68]. Compared to chemical fertilizers, composts increase the content of soil-available nitrogen (N) and soil organic carbon (SOC) and improve soil fertility [69]. Furthermore, using composts can decrease eutrophication risks caused by the excessive use of chemical fertilizers and the subsequent discharge into surface waters [25]. Because all composts included HSs, their use provides an advantage over using mineral fertilizers alone.

## 5. Conclusions

A variety of organic materials can be processed with different treatments (windrow composting, insect frass formation, vermicomposting, etc.) and durations, which all influence the properties of the final product. The present research indicates that, regardless of the input organic waste and composting technology, all studied organic fertilizers are a valuable source of HSs, including HA and FA. The presence of HSs is an advantage over mineral fertilizers alone. All samples exhibited a fluorescence EEM peak of FA associated with polysaccharides. The HA peak dominated in composts comprised of animal by-products, such as fish waste, horse manure, and kitchen biowaste. The core structure of humic acids in *H. illucens* larvae frass differed from other treatments.

Compost quality was assessed commercially by nutrient content and contamination criteria, without considering HSs. To improve soil health and fertility, the concentration and characteristics of HSs could serve as a comprehensive indicator for compost quality evaluation. While HSs act as important biostimulants, their presence and features affect compost's fertilizing value. In the present study, we showed the importance of HS content and properties in a comprehensive evaluation of compost quality.

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## Appendix A

**Table A1.** "Indicators to be measured and presented." from the Regulation of the Republic of Estonia. "Requirements for production of compost from biodegradable waste" RT I, 18.12.2020, 23 2021. issued by the Minister of the Environment.

| Indicator | Parameter | Measurement Unit | Assessment Result |
|---|---|---|---|
| Improvement of soil qualities | Organic substance (OM) | [% in dry matter] | $\geq$15% presentation |
| | Total nitrogen (N) | [% in dry matter] | presentation |
| Fertilising qualities | Total phosphorus (P) | [% in dry matter] | presentation |
| | Total potassium (K) | [% in dry matter] | presentation |
| | Maximum size of particles | [mm] | presentation |
| | Overall density | [g/L] | presentation |
| Material qualities | Water content | [g/L] | |
| | Salinity/conductivity | [mS/m] | presentation |
| | pH value | | presentation |

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# CURRICULUM VITAE


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**Education:**

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| 2018–... | Estonian University of Life Sciences, Institute of Agricultural Sciences, PhD studies in Agricultural Sciences |
| 2014–2016 | University of Tartu, MSc studies in Environmental Technology |
| 2011–2014 | University of Tartu, BSc studies in Environmental Technology |

**Employment:**

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| 01.09.2021–31.01.2023 | Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Soil Science, Junior Researcher |
| 15.01.2019–30.06.2020 | Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, Specialist |

**Professional training:**

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| 02.04.2019–06.04.2019 | Bio-waste recycling and composting study tour in Austria. Organised by Florian Amlinger. |
| 29.04.2019–03.05.2019 | Bio-waste collection, pre-treatment, composting/anaerobic treatment in Milano and bio-waste collection and success story in Contarina. Organised by Novamont S.p.A. |

**Research interests:** Bio-waste recycling, composting, compost quality

**Funding and projects:**

| | |
|---|---|
| 01.01.2022–31.12.2025 | PM220079PKMD "Lambavilla kui olulise bioressursi väärindamise võimalused põllumajanduslikeks toodeteks"; Principal Investigator: Merrit Shanskiy; Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Soil Science; Financier: Estonian University of Life Sciences |
| 01.01.2019–31.12.2022 | PM180261MIMV "Biojäätmetest kõrge lisandväärtusega toodeteni - MIMV"; Principal Investigator: Mait Kriipsalu; Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, Chair of Rural Building and Water Management; Financier: Estonian University of Life Sciences |
| 08.10.2019–07.10.2021 | F190124PKKH (811019790010) "Ogakärblase Hermetia illucens vastsete kasutamine tarneahelast välja mineva kalajäätme taasutiliseerimiseks"; Principal Investigator: Rihard Reissaar; Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences, Chair of Hydrobiology and Fishery; Financier: Estonian Agricultural Registers and Information Board |
| 01.10.2019–21.12.2020 | L190203MIMV "Värska kasutatud ravimuda ja kohaliku biomassi kasutamine kasvusubstraadina"; Principal Investigator: Mait Kriipsalu; Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, Chair of Rural Building and Water Management; Financier: Union of Rural Municipalities of Setomaa |

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|---|---|
| 01.06.2018−31.08.2020 | T180062MIMV (15238) “Applied research in water companies to optimize windrow composting technology for sewage sludge to apply end-of-waste regulation”; Principal Investigator: Mait Kriipsalu; Estonian University of Life Sciences, Institute of Forestry and Rural Engineering, Chair of Rural Building and Water Management; Financier: Environmental Investment Center |
| 28.07.2017–27.07.2020 | T170109PKLJ (811017780003) “Väheväärtusliku peenkala väärindamine “; Principal Investigator: Maidu Silm; Estonian University of Life Sciences, Institute of Agricultural and Environmental Sciences; Financier: Estonian Agricultural Registers and Information Board |

# ELULOOKIRJELDUS


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|---|---|
| 2018–... | Eesti Maaülikool, Põllumajandus- ja Keskkonnainstituut, PhD õpingud Põllumajanduse õppekaval |
| 2014–2016 | Tartu Ülikool, MSc, Keskkonnatehnoloogia eriala |
| 2011–2014 | Tartu Ülikool, BSc, Keskkonnatehnoloogia eriala |

**Teenistuskäik:**

| | |
|---|---|
| 01.09.2021–31.01.2023 | Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Mullateaduse õppetool, Nooremteadur |
| 15.01.2019–30.06.2020 | Eesti Maaülikool, Metsandus- ja maaehitusinstituut, Spetsialist |

**Enesetäiendus ja koolitused:**

| | |
|---|---|
| 02.04.2019–06.04.2019 | Biojäätmete käitlemise ja kompostimise õppereis Austrias. Korraldaja Florian Amlinger. |
| 29.04.2019 –03.05.2019 | Biojäätmete kogumine, eeltöötlus, kompostimine/anaeroobne töötlemine Milanos ning biojäätmete kogumine ja edulugu Contarinas. Korraldaja Novamont S.p.A. |

**Teadustöö põhisuunad:** Biojäätmete käitlemine, kompostimine, komposti kvaliteet

**Uurimisprojektides osalemine:**

| | |
|---|---|
| 01.01.2022−31.12.2025 | PM220079PKMD "Lambavilla kui olulise bioressursi väärindamise võimalused põllumajanduslikeks toodeteks"; Vastutav täitja: Merrit Shanskiy; Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Mullateaduse õppetool; Finantseerija: Eesti Maaülikool. |
| 01.01.2019−31.12.2022 | PM180261MIMV "Biojäätmetest kõrge lisandväärtusega toodeteni – MIMV"; Vastutav täitja: Mait Kriipsalu; Eesti Maaülikool, Metsandus- ja maaehitusinstituut, Maaehituse ja veemajanduse õppetool; Finantseerija: Eesti Maaülikool. |
| 08.10.2019−07.10.2021 | F190124PKKH (811019790010) "Ogakärblase Hermetia illucens vastsete kasutamine tarneahelast välja mineva kalajäätme taasutiliseerimiseks"; Vastutav täitja: Rihard Reissaar; Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut, Hüdrobioloogia ja kalanduse õppetool; Finantseerija: Põllumajanduse Registrite ja Informatsiooni Amet. |
| 01.10.2019−21.12.2020 | L190203MIMV "Värska kasutatud ravimuda ja kohaliku biomassi kasutamine kasvusubstraadina"; Vastutav täitja: Mait Kriipsalu; Eesti Maaülikool, Metsandus- ja maaehitusinstituut, Maaehituse ja veemajanduse õppetool; Finantseerija: MTÜ Setomaa Valdade Liit. |
| 01.06.2018−31.08.2020 | T180062MIMV (15238) "Rakendusuuring vee-ettevõtetes aunkompostimistehnoloogia optimeerimiseks reoveesette jäätmestaatuse lakkamiseks"; Vastutav täitja: Mait Kriipsalu; Eesti Maaülikool, Metsandus- ja maaehitusinstituut, Maaehituse ja veemajanduse õppetool; Finantseerija: SA Keskkonnainvesteeringute Keskus. |

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|---|---|
| 28.07.2017–27.07.2020 | T170109PKLJ (811017780003) "Väheväärtusliku peenkala väärindamine"; Vastutav täitja: Maidu Silm; Eesti Maaülikool, Põllumajandus- ja keskkonnainstituut; Finantseerija: Põllumajanduse Registrite ja Informatsiooni Amet. |

# LIST OF PUBLICATIONS

## Articles indexed by Thomson Reuters Web of Science or Scopus (1.1)


1. Lanno, M., Silm, M., Shanskiy, M., Kisand, A., Orupõld, K., & Kriipsalu, M. (2020). Open windrow composting of fish waste in Estonia. Agronomy Research, 18 (4), 2465−2477. DOI: 10.15159/AR.20.194.
2. Lanno, M., Kriipsalu, M., Shanskiy, M., Silm, M., & Kisand, A. (2021). Distribution of Phosphorus Forms Depends on Compost Source Material. Resources, 10 (10), ARTN 102. DOI: 10.3390/resources10100102.
3. Lanno, M., Klavins, M., Purmalis, O., Shanskiy, M., Kisand, A., & Kriipsalu, M. (2022). Properties of Humic Substances in Composts Comprised of Different Organic Source Material. *Agriculture*, *12*(11), 1797.


## Conference abstracts (5.2)


1. Sepp, M.; Kisand, A.; Shanskiy, M.; Silm, M.; & Kriipsalu, M. (2018). Composting of fish waste. Linnaeus Eco-Tech, 73−73.
2. Sepp, M.; Kisand, A.; Shanskiy, M.; Silm, M.; & Kriipsalu, M. (2019). Comparison of Nutrient Bioavailability in Fish Compost and in Alternative Composts. *21: EGU General Assembly 2019 Vienna Austria 7-12 April 2019*. Geophysical Research Abstracts.
3. Lanno, M., Kriipsalu, M., Klavins, M., Purmalis, O., Kisand, A., & Shanskiy, M. (2022). Bioavailable phosphorus and humic substances in compost–effect on plant growth. *Linnaeus Eco-Tech*.

# VIIS VIIMAST KAITSMIST

**MIHKEL MÄESAAR**
PREVALENCE AND COUNTS OF *LISTERIA MONOCYTOGENES* AND *CAMPYLOBACTER* SPP. IN FOOD AND MOLECULAR CHARACTERISATION OF THE ISOLATES IN ESTONIA
*LISTERIA MONOCYTOGENES*'E JA *CAMPYLOBACTER* SPP. LEVIMUS JA ARVUKUS TOIDUS NING TÜVEDE MOLEKULAARNE ISELOOMUSTUS EESTIS
**Professor Mati Roasto**
14. detsember 2022

**MARILI VESTER**
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**Professor Rein Drenkhan**
10. jaanuar 2023

**ELISABETH DORBEK-KOLIN**
CRYPTOSPORIDIUM SPP. PREVALENCE, RELATIONSHIP WITH THE GENERAL INFLAMMATORY RESPONSE, FAECAL MICROBIOTA AND HALOFUGINONE LACTATE TREATMENT IN CALVES
KRÜPTOSPORIIDIDE LEVIMUS, SEOS ÜLDISE PÕLETIKUVASTUSE, ROOJA MIKROBIOOTA JA HALOFUGINOON LAKTAADI RAVIGA VASIKATEL
**Professor Toomas Orro**
10. veebruar 2023

**MARII RASVA**
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PÕLLUMAJANDUSMAA KASUTUSE MUUTUSED EESTIS: TASAKAALUSTATUD MAAKASUTUSE TAGAMISE VÕIMALUSED
**Kaasprofessor Evelin Jürgenson ja kaasprofessor Aive Liibusk**
9. märts 2023

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LIQUID FUELS AIR-FUEL MIXTURE PROCESSING SYSTEM WITH SPARK IGNITION ENGINE FOR USE IN GENERATORS IN A CRISIS SITUATION
VEDELKÜTUSTEST MOODUSTATUD KÜTTESEGU TÖÖTLEMISE SÜSTEEM SÄDESÜÜTEGA MOOTORGENERAATORITES KASUTAMISEKS KRIISI OLUKORRAS
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